# EFFECTS OF SPECIFIC FACTORS ON THE FATTY ACID COMPOSITION OF *Streptococcus lactis*

By

Timothy Alan Kral

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1978

## ACKNOWLEDGMENTS

The author is extremely grateful to Dr. Kenneth L. Smith for his guidance and support during the period of this research.

Gratitude is also expressed to Drs. Kermit C. Bachman, Arnold S. Bleiweis, and Paul H. Smith for serving as members of the supervisory committee, and to all the members of the Department of Microbiology and Cell Science and the Dairy Science Department for their assistance.

Special thanks go to the author's parents, Mr. and Mrs. George E. Kral for their support, encouragement, and love. This work is dedicated to them.

TABLE OF CONTENTS

ACKNOWLEDGMENTS . . . . . ii

ABSTRACT . . . . . v

INTRODUCTION . . . . . 1

ORGANISM . . . . . 5

COMMON METHODS . . . . . 7
Growth Conditions . . . . . 7
Lipid Extraction and Esterification . . . . . 9
Fatty Acid Methyl Ester Analysis . . . . . 9

CHAPTER I. EFFECTS OF GROWTH TEMPERATURE ON THE FATTY ACID COMPOSITION OF *Streptococcus lactis* . . . . . 10
Literature Review . . . . . 10
Additional Methods . . . . . 12
Results . . . . . 13
Discussion . . . . . 31

CHAPTER II. EFFECTS OF pH ON THE FATTY ACID COMPOSITION OF *Streptococcus lactis* . . . . . 34
Literature Review . . . . . 34
Additional Methods . . . . . 35
Results . . . . . 35
Discussion . . . . . 38

CHAPTER III. EFFECTS OF GROWTH PHASE ON THE FATTY ACID COMPOSITION OF *Streptococcus lactis* . . . . . 55
Literature Review . . . . . 55
Additional Methods . . . . . 56
Results . . . . . 56
Discussion . . . . . 59

CHAPTER IV. EFFECTS OF VARIOUS CARBOHYDRATE SOURCES ON THE FATTY ACID COMPOSITION OF *Streptococcus lactis* . . . . . 79
Literature Review . . . . . 79
Additional Methods . . . . . 79
Results . . . . . 80
Discussion . . . . . 97

CHAPTER V. EFFECTS OF CITRATE ON THE FATTY ACID COMPOSITION OF *Streptococcus lactis* . . . . . 98
Literature Review . . . . . 98
Additional Methods . . . . . 98
Results . . . . . 99
Discussion . . . . . .117

CHAPTER VI. EFFECTS OF ADDED FERMENTATION PRODUCTS ON THE FATTY ACID COMPOSITION OF *Streptococcus lactis* . . . . .118
Literature Review . . . . . .118
Additional Methods . . . . . .119
Results . . . . . .119
Discussion . . . . . .137

GENERAL DISCUSSION . . . . . .138

BIBLIOGRAPHY . . . . . .142

BIOGRAPHICAL SKETCH . . . . . .146

Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

EFFECTS OF SPECIFIC FACTORS ON THE
FATTY ACID COMPOSITION OF *Streptococcus lactis*

By

Timothy Alan Kral

August 1978

Chairman: Kenneth L. Smith
Major Department: Microbiology and Cell Science

The effects of temperature, pH, growth phase, carbohydrate source, citrate, and fermentation products on the fatty acid composition of *Streptococcus lactis* ATCC 12929 were investigated. Organisms were grown in a defined medium at constant temperature and pH. Optical densities of cultures were measured throughout growth to determine doubling times. At the appropriate time during growth, cells were harvested, lipids were extracted, and fatty acids were converted to methyl esters. The methyl esters were analyzed via gas chromatography.

The major fatty acids found were 14:0, 16:0, 16:1, 18:0, 18:1, Δ19:0, and 21:0. The most dramatic differences occurred in the 18:1 and Δ19:0 fatty acids under all conditions tested. At a temperature of 30°C, 18:1 levels were their highest while Δ19:0 levels were their lowest. As the temperature became more adverse in either direction, the Δ19:0 fatty acid increased at the expense of decreases in the 18:1 fatty acid.

A pH of 6.5 resulted in a high level of 18:1 fatty acid with a very low level of the Δ19:0 fatty acid. At low pH values, the Δ19:0 levels again increased with decreasing levels of 18:1 fatty acid. At higher pH values, the 18:1 content decreased while the Δ19:0 content remained low. Levels of the 18:1 fatty acid also decreased with concomitant increases in the Δ19:0 fatty acid as cells proceeded from exponential to stationary phase of growth.

Growth on various carbohydrate sources demonstrated variations in 18:1 and Δ19:0 fatty acid levels with no quantitative correlation with growth rate. Varying concentrations of citrate caused insignificant changes in the levels of these two fatty acids. Only at a level of citrate ten times that normally found in the growth medium were levels of Δ19:0 elevated and 18:1 levels depressed. In all cases of added fermentation products, higher concentrations of those products caused lower levels of the 18:1 fatty acid and higher levels of the Δ19:0 fatty acid.

In general, when the organisms seemed to be most content, the levels of 18:1 fatty acid would be high while levels of Δ19:0 fatty acid would be low. As conditions became more adverse, the Δ19:0 fatty acid increased at the expense of decreasing 18:1 fatty acid levels, except at pH values above 6.5 where Δ19:0 levels remained low. Although a gross relationship between growth rate and Δ19:0 fatty acid production seemed apparent, mathematical models tested could demonstrate no quantitative correlation.

Chairman

# INTRODUCTION

Every living organism differs from its environment in both its physical properties and its chemical composition. In order to maintain these differences, some structure must exist which separates the organism from its surroundings. This structure is the cell membrane. But separating the cell from its environment is only one role of the cell membrane. Since the cell must interact with its environment, the cell membrane must allow selective interaction between the interior of the cell and its surroundings (11).

Since biological membranes play a crucial role in almost all cellular phenomena, an understanding of the molecular organization of the membrane is necessary. The principal components of membranes are lipids and proteins. Carbohydrates may be present in small amounts, usually from none to less than 10% of the total mass of the membrane (1). The ratio of lipid to protein varies greatly from one type of cell to another. In the myelin membrane sheathing of nerve fibers the proportion of lipid to protein is approximately nine to one. This is probably due to the fact that the myelin membrane functions as an insulator. Mitochondrial membranes, on the other hand, have a ratio of about one to one. This relatively high protein ratio is accounted for by the many enzyme systems associated with the mitochondrion (4).

Proteins play a variety of roles in membranes. They may contribute to the structural integrity of the membrane, they can act as enzymes or they can function as pumps, transporting molecules in and out of

the cell (2). It is believed that it is the interaction of these proteins with the lipid components which gives the membrane its characteristics and vital properties.

A necessary step toward the understanding of membranes is the understanding of the lipid component. Organisms which have many advantages for studying membrane lipids are the bacteria. They have no intracellular membranes, and their lipid compositions are much simpler than the compositions of eucaryotic cells (8). Of the bacteria, the gram positives are less complex than the gram negatives since gram positives have one cytoplasmic membrane while gram negatives have an inner and outer membrane (33).

Phospholipids are the principal lipid constituents of bacterial membranes. The common lipids found are phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl glycerol and cardiolipin (41). While most gram negatives are composed almost entirely of phospholipids, most gram positives also include varying proportions of glyco- and neutral lipids. Sphingolipids and cholesterol are absent from bacteria (41). Membrane lipids differ among themselves not only in polar head groups, but also in the identity of their constituent fatty acids. Phospholipid and glycolipid molecules may contain any two of more than half a dozen fatty acids while a neutral lipid may contain any three (4).

Phospholipids and glycolipids, the two most common lipids in gram positive bacteria, have a head and two tails, the head being the phosphate moiety or carbohydrate respectively, and the tails being the fatty acid chains. The tails, being hydrophobic, tend to point away from water, while the head groups, being hydrophilic, are attracted to water. Lipids in membranes are arranged to accommodate this

amphipathic character. They form a bilayer, two layers back to back, with their hydrophilic heads constituting the outer surfaces and their hydrophobic tails buried in the interior (2). This bilayer structure results in maximizing both hydrophobic and hydrophilic interactions which contribute to the stabilization of the membrane (44).

These thermodynamic considerations along with experimental results accommodate a mosaic membrane structure in which globular proteins alternate with sections of the bilayer in the cross-section of the membrane. These globular proteins, being amphipathic to varying degrees, would fit well into the bilayer, with hydrophobic amino acid portions associating with the hydrophobic fatty acid interior and the hydrophilic portions associating with the hydrated surface (44).

This mosaic membrane structure is by no means static. Both the lipids and the proteins have considerable freedom of movement (2). This is due to the fact that under physiological conditions, the lipids are in a fluid rather than a crystalline state (44). This fluid nature of membranes has been established by application of both x-ray diffraction and differential scanning calorimetry to bacterial membranes. These techniques have shown that the membrane can pass through a thermal phase transition. At temperatures below the transition, the fatty acids are in a relatively rigid crystalline state while at temperatures above the transition, the fatty acids assume a more fluid random structure. At temperatures in between, both fluid and solid lipid phases exist. The temperature range of this transition is determined largely by the fatty acid composition of the membrane. Chain length, degree and position of unsaturation, and the configuration of the unsaturation (cis or trans) of the fatty acids are important factors. Longer chain lengths lead to greater stability of the crystalline

state and thus to higher transition temperatures (4,41). Membranes formed from lipids containing fully saturated linear fatty acids also have higher transition temperatures. Lipids with cis-unsaturated fatty acids undergo transitions at much lower temperatures due to the instability created by the discontinuity in the regular array of fatty acid side chains (18,41).

Organisms have a sufficient number of double bonds in their lipids to maintain the bilayer membrane in a lipid crystalline or fluid state at growth temperatures (18,41). Lipids of some organisms contain, instead of double bonds, cyclopropyl groups or branched chains which produce kinks in the regular array of fatty acid side chains, and serve to maintain membrane fluidity (18). This is especially true in gram positive bacteria (23,34,35). This seems to be necessary since such processes as membrane biogenesis, transmembrane transport, and exo- and endocytosis can occur only when the membrane exhibits sufficient fluidity. If a membrane is too fluid, it may lack integrity. Therefore, living organisms have evolved mechanisms to regulate the degree of unsaturation as well as chain length, ensuring a proper degree of membrane fluidity (6,41).

The regulation of proper fatty acid composition when the organism is subjected to a variety of environmental conditions is the theme of this work. The organism chosen for this investigation was *Streptococcus lactis* ATCC 12929, a gram positive bacterium. Six environmental parameters were chosen: temperature, pH, growth phase, carbohydrate source, presence of citrate, and presence of added fermentation products.

## ORGANISM

*S. lactis* ATCC 12929 is a gram positive coccus which typically occurs in pairs. Antigenically, it is a Lancefield Group N bacterium. Its growth range is from below 10°C to 40°C with an optimum at about 30°C. Its pH range is from about 4.2 to 9.2. In broth culture, it grows microaerophilically, while on milk agar it forms pinpoint size colonies. *S. lactis* is probably of plant origin, but it is found commonly as a contaminant in milk and is used industrially in the production of dairy products such as buttermilk (12).

*S. lactis* ATCC 12929 contains a variety of fatty acids, some of which occur in relatively large quantities, while others are only found in trace amounts. These are listed in Table 1. This dissertation will be concerned only with the major fatty acids.

Table 1. Fatty acids found in *S. lactis* ATCC 12929.

| Major fatty acids (>1.0%) | Trace fatty acids (<1.0%) |
|---|---|
| 14:0 | 12:0 |
| 16:0 | 12:1 |
| 16:1 | 14:1 |
| 18:0 | iso 16:0 |
| 18:1 | anteiso 21:0 |
| Δ19:0 | 22:0 |
| 21:0 | |

## COMMON METHODS

The methods mentioned here were used in all of the experiments with some alterations in individual experiments. Those alterations will be noted when necessary.

### Growth Conditions

*S. lactis* ATCC 12929 was propagated in a defined medium (40) supplemented with 1.0% glucose. A 2.0 ml inoculum of an overnight culture (30°C) was added to 200 ml of the same medium in a 1.0 l jar. Immediately prior to inoculation, the medium was sterilized by membrane filtration. The jar was kept immersed in a constant temperature water bath held at 30°C. Constant agitation was maintained by a magnetic stirrer in the jar. Immersed in the medium were two pH electrodes which were connected to a Versatrol Control Unit (Assembly Products, Inc.) through a pH meter (Beckman). The Versatrol controlled a Model 610 Randolf Pump equipped with a variable speed control unit. As the pH decreased due to acid production by the bacteria, the Versatrol Control Unit automatically activated the pump which fed sterile 3N KOH into the growth vessel. In this manner, the pH was maintained at 6.5. An aquarium pump forced air through two sterile cotton filters into the growth vessel, maintaining a constant positive pressure, thus allowing for a contaminant-free system. Figure 1 shows a diagram of this growth setup. Culture purity was verified at the end of each experiment by the gram stain technique. Samples were

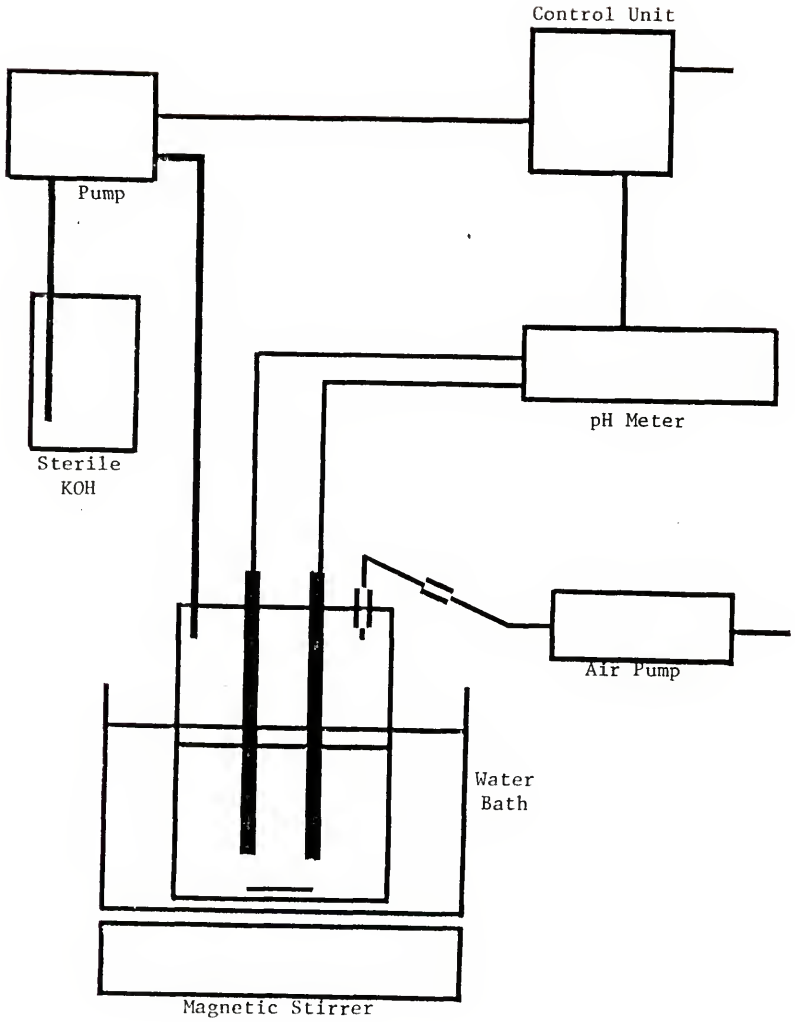



Figure 1. Diagram of the growth setup.

taken from the cultures at various times during growth, and optical densities were measured at 540 nm using a Spectronic 20 (Bausch and Lomb) in order to construct growth curves and thereby determine doubling times. When the cultures reached mid to late exponential phase, they were immediately inactivated by addition of 5.0% trichloroacetic acid (w/v).

## Lipid Extraction and Esterification

The trichloroacetic acid-treated samples were pelleted in a Sorvall Superspeed RC2-B centrifuge at 12,000 X g for 10 minutes. The pellet was extracted with chloroform:methanol (2:1 v/v) according to the method of Kanfer and Kennedy (24). The extract was evaporated under nitrogen gas and then transesterified with 2.0% $H_2SO_4$ in methanol (w/v) for 2 hours at 80°C. Fatty acid methyl esters were extracted twice into pentane and analyzed via gas chromatography.

## Fatty Acid Methyl Ester Analysis

The pentane extracts were concentrated using nitrogen gas and 0.5 µl aliquots were injected into a Series 810 F & M Research Chromatograph equipped with a flame ionization detector and a 183 cm by 0.64 cm stainless steel column. The column was packed with 10% EGSS-X on Gas Chrom Q (100/120 mesh). The gas chromatograph was run isothermally at 180°C. Nitrogen gas was used as the carrier at a flow rate of 30 ml/min. Fatty acid methyl esters were identified by comparison with authentic standards (Applied Sciences Laboratory) as well as by relative weight percentages. Relative percentages of fatty acid methyl esters were determined by the product of retention time and peak height (3).

# CHAPTER I
# EFFECTS OF TEMPERATURE ON THE FATTY ACID COMPOSITION OF *S. lactis*

## Literature Review

As mentioned in the introduction, the mosaic membrane structure is not static. The lipids and proteins have considerable freedom of movement due to the fact that under physiological conditions, the lipids are in a fluid rather than a crystalline state (2,44). With regard to temperature, the general rule is that as the growth temperature is lowered, the composition of the fatty acid chains is modified in the direction of the lower average melting points. This adjustment makes it less likely that all of the lipids will become frozen, a condition that would interfere with vital functions (27). Changes in the physical state of the lipids affect biological activities in membranes at two characteristic temperatures. Below the lower temperature, all the membrane lipids are in a solid state. Above the higher temperature, all of the membrane lipids are in a fluid state (49). At temperatures between these low and higher temperatures, both fluid and solid phases exist. The temperature range of this transition is determined largely by the fatty acid composition of the membrane (41). Membranes containing fully saturated linear fatty acids have higher transition temperatures whereas membranes containing cis-unsaturated fatty acids undergo transitions at much lower temperatures due to the instability created by the discontinuity in the regular array of fatty acid side chains (18,41).

In gram negative bacteria, as the temperature is lowered, there is usually an increased proportion of unsaturated fatty acids at the expense of saturated fatty acids (27). Marr and Ingraham (36) demonstrated that the proportion of unsaturated fatty acids in *Escherichia coli* increased continuously as the growth temperature was decreased in both minimal and complex media. The proportion of cyclopropane fatty acids also decreased at lower temperatures. Similar changes were observed when *Serratia marcescens* was grown at 30°C and 10°C (26).

In *Pseudomonas fluorescens*, degree of fatty acid saturation varied to some extent but not over the whole growth temperature range. A minimum degree of saturation was obtained at about 10°C and further decreases of the growth temperature did not significantly alter the fatty acid composition. *P. fluorescens* controls its fatty acid composition only over the lower third of its growth temperature range by increasing its relative amount of unsaturated fatty acids with decreasing temperature. A precise degree of saturation apparently is not required for normal growth at higher temperatures (10,19).

In some gram positive bacteria there are increased amounts of lower melting branched chain fatty acids at the expense of the higher melting branched and straight chain fatty acids at lower growth temperatures. In addition, a fatty acid desaturating system is induced in some bacilli at lower growth temperatures resulting in lower melting unsaturated fatty acids (27). In both *Bacillus caldolyticus* and *Bacillus caldotenax*, decreased growth temperature caused an increase in iso 15:0 and a decrease in iso 17:0. The iso 17:0 would correspond to a more saturated fatty acid due to its higher melting point. Also observed was an increase in the iso 16:0 fatty acid corresponding to a decrease

in the 16:0 fatty acid (48). Even in *E. coli*, a gram negative bacterium, the cis-unsaturated fatty acids can be totally replaced by branched chain fatty acids bearing bulky substituents which disrupt membrane packing (42).

Many gram positive bacteria, however, have fatty acid profiles similar to gram negative bacteria and behave accordingly with respect to changes in growth temperature. *Clostridium butyricum* shows a general increase in the proportions of unsaturated and cyclopropane fatty acids when the temperature is lowered from 37°C to 25°C (27). Decreases in growth temperature have also resulted in increased ratios of unsaturated to saturated fatty acids in *Streptococcus mutans* (17).

The fatty acid composition of *S. lactis* 527, grown at two different temperatures (10°C and 30°C), has also been investigated. Results showed that unsaturated fatty acids increased while lactobacillic acid (Δ19:0) decreased at 10°C compared to 30°C in the neutral lipid fraction. Complex lipids showed little changes in fatty acids with respect to temperature differences. The investigators postulated that the formation of the lactobacillic acid might have been delayed or inhibited at the lower temperatures (47).

Lactobacillic acid has also been shown to decrease with lower growth temperature in *Streptococcus faecalis*. The final percentage of lactobacillic acid in cells grown at 27, 37, and 47°C was 15.9, 22.6, and 36.4% respectively (22).

## Additional Methods

Organisms were grown at 10, 15, 20, 25, 30, 35, and 39°C. Growth curves were plotted and doubling times were determined for growth at each temperature. This experiment was run in triplicate and an analysis of variance was applied to the data.

## Results

Doubling times for growth at the various temperatures are listed in Table 2. The fastest growth occurred at 35°C under the given set of conditions.

Table 3 lists the percentages of the fatty acids at each temperature tested. Except for the 18:1 and Δ19:0 fatty acids, the remaining fatty acids showed relatively small differences throughout the temperature range. The 14:0 content remained constant at 10°, 15°, and 20°C, and then showed a gradual rise of about 6 percentage points from 20° to 39°C. A gradual rise of 5 percentage points occurred in the 16:0 content from 10° to 35°C at which point it remained constant up to 39°C. The 16:1, 18:0, and 21:0 fatty acids showed slight increases and decreases of no more than 3 percentage points throughout the range tested.

Dramatic differences appeared in the 18:1 and Δ19:0 fatty acid contents. An increase of approximately 20 percentage points occurred in the 18:1 fatty acid going from 10° to 30°C followed by a sharp decrease of 30 percentage points from 30° to 39°C. The opposite effect was observed in the Δ19:0 fatty acid. Going from 10° to 30°C, a drop of 23 percentage points occurred followed by a rise of 20 percentage points when the temperature increased from 30° to 39°C. These differences are graphically represented in Figures 2-8.

F-values and significance of differences are shown in Table 4. Significant differences were seen in all of the fatty acids except in the 18:0 fatty acid.

Table 2. Doubling times of *S. lactis* grown at various temperatures.

| Temperature (°C) | Doubling time (min) |
|---|---|
| 10 | 3800 |
| 15 | 2100 |
| 20 | 465 |
| 25 | 190 |
| 30 | 75 |
| 35 | 70 |
| 39 | 312 |

Table 3. Fatty acid percentages from *S. lactis* grown at various temperatures.

| Fatty acid | Temperature (°C) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 10 | 15 | 20 | 25 | 30 | 35 | 39 |
| 14:0 | 1.9 | 1.7 | 1.9 | 2.7 | 3.5 | 5.0 | 7.1 |
| 16:0 | 27.8 | 29.3 | 30.3 | 31.6 | 33.0 | 33.7 | 33.1 |
| 16:1 | 5.5 | 4.6 | 3.8 | 3.6 | 2.7 | 3.1 | 4.4 |
| 18:0 | 1.7 | 2.5 | 1.7 | 2.0 | 2.9 | 2.0 | 2.4 |
| 18:1 | 28.2 | 35.3 | 41.7 | 42.6 | 46.7 | 38.1 | 18.7 |
| Δ19:0 | 30.3 | 23.1 | 16.9 | 14.0 | 8.1 | 12.7 | 28.2 |
| 21:0 | 2.9 | 1.9 | 1.5 | 2.0 | 1.6 | 2.7 | 3.6 |

Figure 2. Percentages of the 14:0 fatty acid from *S. lactis* grown at various temperatures.

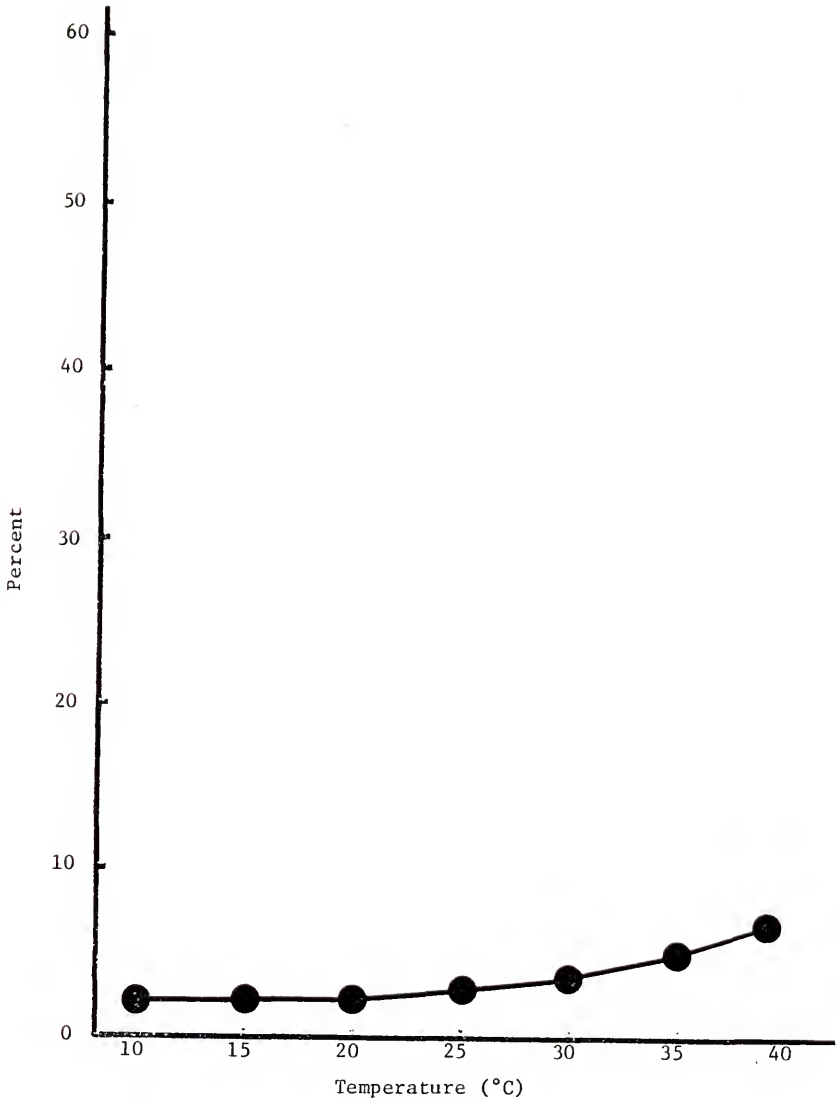

60
50
40
30
20
10
0
Percent
10
15
20
25
30
35
40
Temperature (°C)

Figure 3. Percentages of the 16:0 fatty acid from *S. lactis* grown at various temperatures.

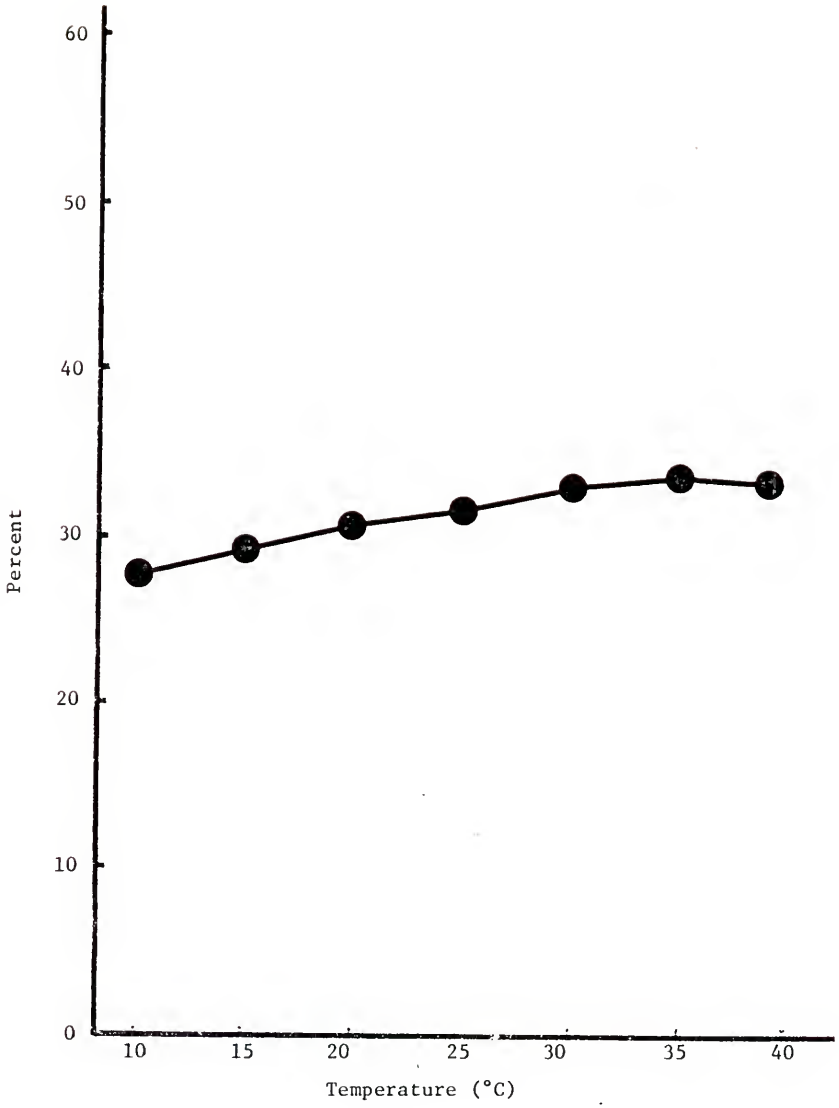

60
50
40
30
20
10
0
Percent
10
15
20
25
30
35
40
Temperature (°C)

Figure 4. Percentages of the 16:1 fatty acid from *S. lactis* grown at various temperatures.

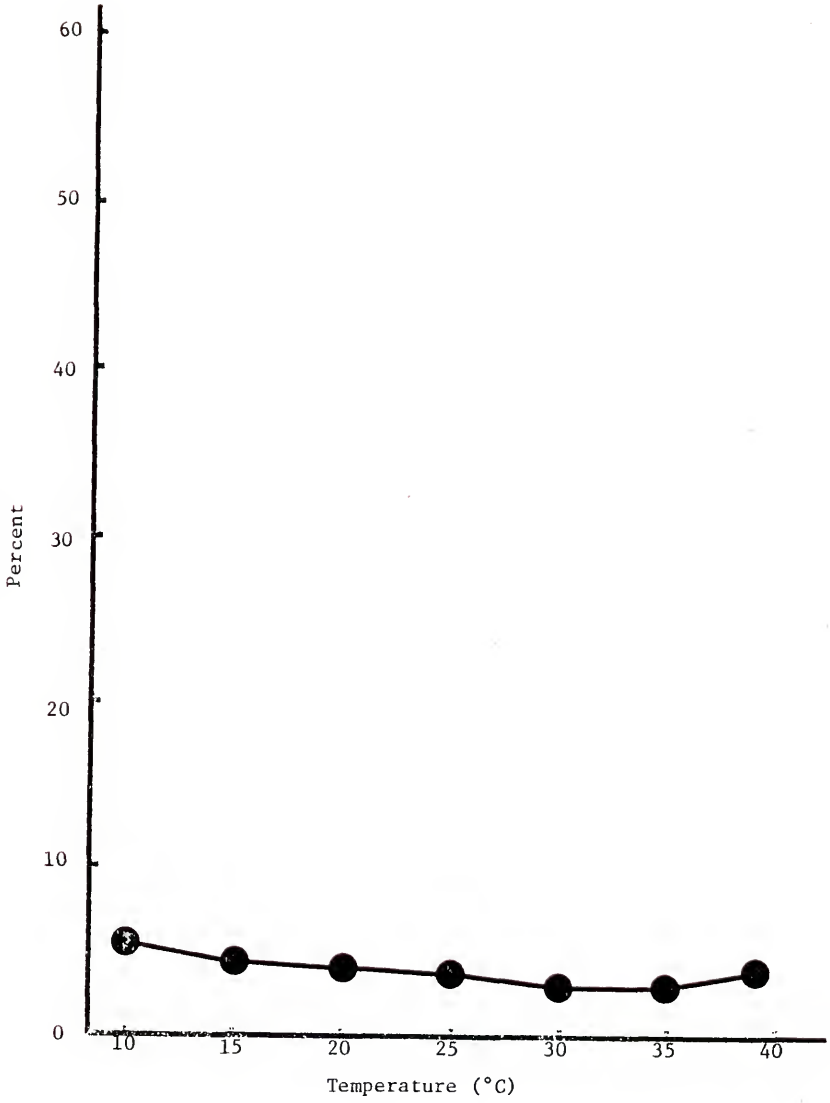

60
50
40
30
20
10
0
Percent
10
15
20
25
30
35
40
Temperature (°C)

Figure 5. Percentages of the 18:0 fatty acid from *S. lactis* grown at various temperatures.

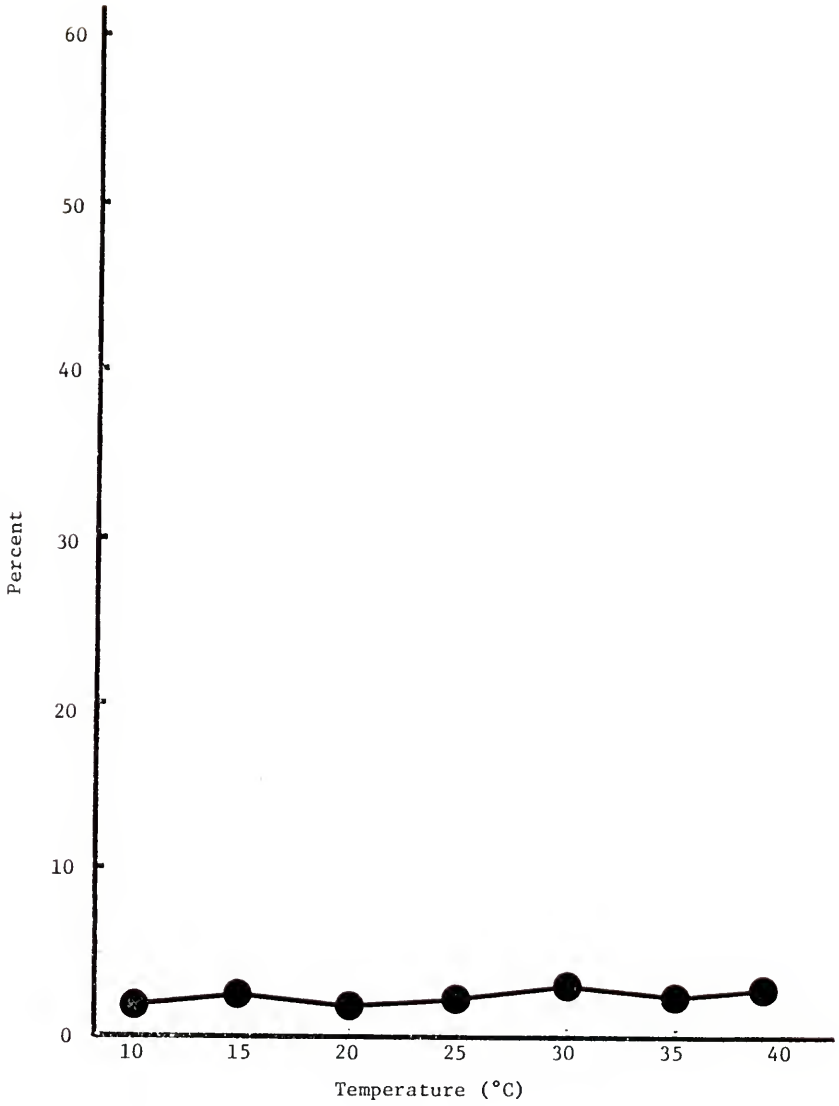

60
50
40
30
20
10
0
Percent
10
15
20
25
30
35
40
Temperature (°C)

Figure 6. Percentages of the 18:1 fatty acid from *S. lactis* grown at various temperatures.

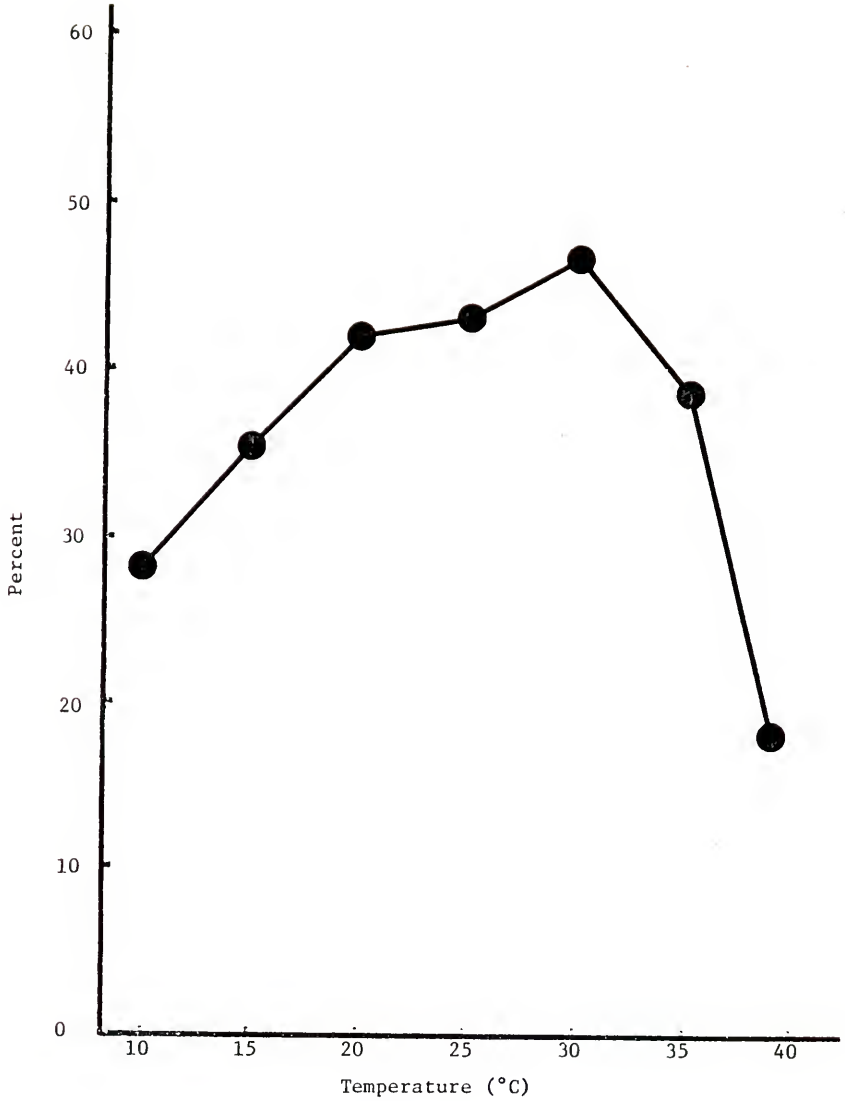

60
50
40
30
20
10
0
Percent
10
15
20
25
30
35
40
Temperature (°C)

Figure 7. Percentages of the Δ19:0 fatty acid from *S. lactis* grown at various temperatures.

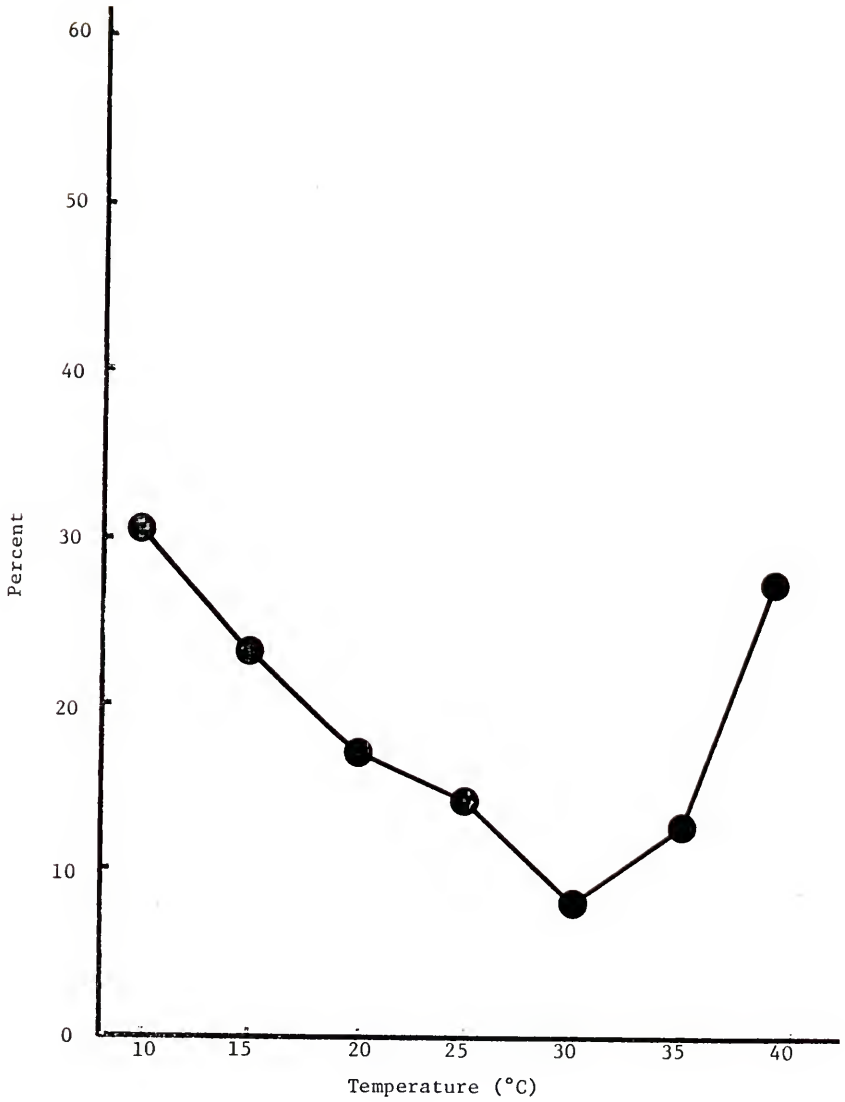

60
50
40
30
20
10
0
Percent
10
15
20
25
30
35
40
Temperature (°C)

Figure 8. Percentages of the 21:0 fatty acid from *S. lactis* grown at various temperatures.

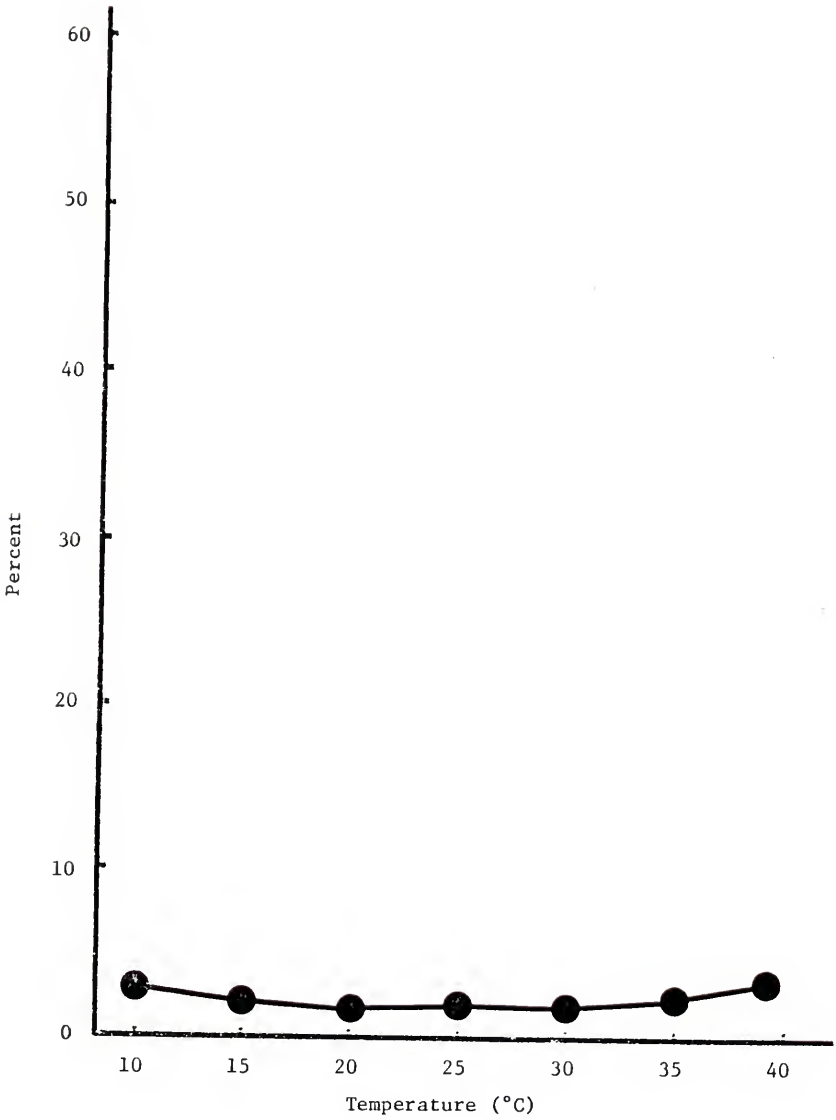

60
50
40
30
20
10
0
Percent
10
15
20
25
30
35
40
Temperature (°C)

Table 4. F-values and significance of differences for temperature experiment.

| Fatty Acid | F-value[a] | Significance[b] |
|---|---|---|
| 14:0 | 58.43 | * |
| 16:0 | 17.37 | * |
| 16:1 | 10.72 | * |
| 18:0 | 2.12 | NS |
| 18:1 | 52.50 | * |
| Δ19:0 | 107.02 | * |
| 21:0 | 11.06 | * |

[a] $F_{.05,6,14} = 2.85$

[b] * = significant; NS = not significant

## Discussion

Four of the seven major fatty acids, 14:0, 16:0, 16:1, and 21:0 showed statistically significant differences, but the maximum range of differences (<6 percentage points) seemed so small that on first analysis, any functional significances seemed unlikely. Only the 18:1 and Δ19:0 fatty acids showed statistically significant differences as well as relatively large numerical differences. The 18:0 fatty acid showed no significant differences at all.

It probably can be assumed that *S. lactis*, as well as all forms of life, must maintain a functional membrane fluidity to maintain the essence of life. If that functional fluidity is indeed derived from the balances of saturated and unsaturated fatty acids in the bilayer of the membrane, then it is difficult to envisage how the dramatic differences in the 18:1 and Δ19:0 fatty acids could account for that level of fluidity. Rather than increase or decrease continuously throughout the temperature range, each achieved a point (30°C) where it changed directions. The 18:1 fatty acid increased from 10 to 30°C and then decreased sharply up to 39°C. The opposite effect is seen in the Δ19:0 fatty acid. There seems to be a balance between the two, which is reasonable since the Δ19:0 fatty acid is derived from the 18:1 fatty acid by the methylation of the 18:1 double bond by S-adenosylmethionine. This reaction is catalyzed by cyclopropane fatty acid synthetase (37,50). Summing up the 18:1 and Δ19:0 percentages at each temperature generates the values seen in Table 5. These sums also represent the amounts of 18:1 fatty acid incorporated into the membrane. As the temperature proceeds from 10 to 39°C, the sums remain constant up to 20°C and then consistently decrease by about 11 percentage points. If the Δ19:0

Table 5. Sums of the 18:1 and Δ19:0 fatty acid percentages at each growth temperature.

| Temperature (°C) | 18:1 | Δ19:0 | 18:1 + Δ19:0 |
|---|---|---|---|
| 10 | 28.2 | 30.3 | 58.5 |
| 15 | 35.3 | 23.1 | 58.4 |
| 20 | 41.7 | 16.9 | 58.6 |
| 25 | 42.6 | 14:0 | 56.6 |
| 30 | 46.7 | 8.1 | 54.8 |
| 35 | 38.1 | 12.7 | 50.8 |
| 39 | 18.7 | 28.2 | 46.9 |

fatty acid is considered comparable to the 18:1 fatty acid in regards to fluidity characteristics due to its cyclopropane ring which would cause some disorder in the fatty acid packing, then the decrease in sums would represent a decrease in total unsaturation which is consistent with the theory mentioned previously. Accordingly, the small increases in the 14:0 and 16:0 fatty acid percentages, approximately 11 percentage points, would both balance the decrease in unsaturation as well as satisfy the theory: with increasing temperature, there is an increase in saturation with an accompanying decrease in unsaturation.

But why do the percentages of the 18:1 and Δ19:0 fatty acids change so drastically? Changes to conserve fluidity obviously could have been more conservative. There must be another reason to explain the differences observed. Both the 18:1 high point and the Δ19:0 low point occur at 30°C. In this experiment, the most rapid growth occurred at 35°C, but the optimum temperature usually quoted for *S. lactis* is around 30°C (12). Whether the optimum growth temperature corresponds to the fastest growth temperature is a matter of semantics. In any case, *S. lactis* seems to grow quite well at 30°C. Assuming that *S. lactis* is "happiest" at 30°C, then temperatures above and below 30°C would be less desirable. This corresponds to the differences observed in the 18:1 and Δ19:0 fatty acids. As the temperature proceeds away from 30°C, the 18:1 content decreases while the Δ19:0 content increases. Since the conversion of 18: to Δ19:0 occurs to the greatest extent when the organisms encounter adverse temperatures, it seems likely that the Δ19:0 fatty acid may be protective in some way.

CHAPTER II
EFFECTS OF pH ON THE FATTY ACID COMPOSITION
OF *S. lactis*

## Literature Review

Very little work has been done correlating fatty acid differences with differences in pH. In 1975, Drucker, Griffith and Melville (16) reported on the effects of pH on the fatty acid profiles of various strains of *S. mutans*. Cells were grown at a series of pH values ranging from 5.8 to 8.3. Below pH 6.5 fatty acid profiles were relatively stable. From pH 6.7 to 7.3 the proportions of 16:1 and 18:0 changed. The 16:1 increased approximately 10 percentage points while the 18:0 decreased about 12. These same fatty acids remained stable from pH 7.3 to 8.3. The 16:0 and 18:1 fatty acids remained relatively constant below pH 7.0, while increasing and decreasing randomly at alkaline pH values. Therefore, the major cellular fatty acids of the strains of *S. mutans* investigated remained fairly constant below pH 6.7, while showing significant differences above pH 6.7 without, however, demonstrating any discernible patterns.

Effects of pH on the fatty acid composition of *Bacillus acidocaldarius* have also been examined. Results indicated that the effects of temperature and pH are interdependent. At pH 2.0, increasing temperature raised the proportion of iso and anteiso fatty acids (as much as 9 percentage points) while at pH 5.0, the effect of increasing temperature was reversed and the proportion of cyclohexyl fatty acids was increased (as much as 22 percentage points) (14).

Studies done with *S. faecalis* showed that low pH was correlated with an increased synthesis of cyclopropane fatty acids (21).

In *E. coli*, below pH 6.2, the percentage of the Δ17:0 fatty acid increased with acidity until at pH 4.5, it was three times that at pH 6.2. There was little change in Δ17:0 above pH 6.2. Below pH 5.5, some Δ19:0 fatty acid also appeared. And there was a decrease in the 16:1 fatty acid with increasing acidity below pH 6.2 (29).

## Additional Methods

The organisms were grown at pH values of 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0. Growth curves were plotted and doubling times determined for growth at each pH. This experiment was run in triplicate and an analysis of variance was applied to the data.

## Results

Table 6 lists the doubling times for growth at each pH tested. A pH of 6.0 seemed to induce the most rapid growth.

Fatty acid percentages for each pH tested are listed in Table 7. All of the fatty acids in this experiment demonstrated differences with the 18:1 and Δ19:0 fatty acids again showing the really dramatic differences. The most constant fatty acid was 16:1 showing a gradual increase of 5 percentage points going from pH 4.5 to 9.0. The 14:0 fatty acid showed an increase of 5 percentage points from pH 5.0 to 6.0, then a slight decrease of 2 percentage points at pH 7.0, and then a gradual increase of 5 percentage points up to pH 9.0. A fairly constant percentage with a 2 percentage point dip at pH 6.5 and an increase of 4 percentage points going from pH 8.5 to 9.0 characterized the 18:0 fatty acid. The 21:0 content also remained constant from pH 5.5 to 9.0, but from 5.5 to 5.0 it increased 7 percentage points and then leveled out

Table 6. Doubling times of *S. lactis* grown at various pH's.

| pH | Doubling time (min,) |
|---|---|
| 4.5 | 155 |
| 5.0 | 115 |
| 5.5 | 80 |
| 6.0 | 77 |
| 6.5 | 85 |
| 7.0 | 95 |
| 7.5 | 146 |
| 8.0 | 173 |
| 8.5 | 456 |
| 9.0 | 740 |

Table 7. Fatty acid percentages from *S. lactis* grown at various pH's

| Fatty acid | pH | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
| 14:0 | 1.0 | 1.9 | 4.8 | 6.0 | 4.7 | 4.0 | 4.5 | 6.0 | 7.1 | 9.1 |
| 16:0 | 37.9 | 34.5 | 32.7 | 29.8 | 28.9 | 30.1 | 29.8 | 33.9 | 36.9 | 37.9 |
| 16:1 | 1.1 | 1.7 | 2.5 | 2.8 | 2.7 | 2.8 | 3.7 | 5.0 | 5.7 | 6.9 |
| 18:0 | 3.6 | 3.6 | 3.5 | 3.4 | 1.7 | 3.3 | 2.1 | 2.6 | 4.0 | 8.1 |
| 18:1 | 5.1 | 12.3 | 35.2 | 42.4 | 51.6 | 48.0 | 46.5 | 41.2 | 37.8 | 31.1 |
| Δ19:0 | 38.3 | 33.4 | 13.3 | 9.5 | 7.2 | 6.8 | 7.8 | 6.4 | 5.5 | 3.1 |
| 21:0 | 10.0 | 9.1 | 2.6 | 2.5 | 2.6 | 2.2 | 2.2 | 2.5 | 1.8 | 1.9 |

going down to pH 4.5. The 16:0 fatty acid decreased 9 percentage points from pH 4.5 to 6.0, remained constant up to pH 7.5, and then increased 9 percentage points up to pH 9.0.

A very large increase of 48 percentage points was observed going from pH 4.5 to 6.5 in the 18:1 fatty acid which then showed a decrease of 20 percentage points as the pH increased to 9.0. The Δ19:0 fatty acid, on the other hand, decreased 25 percentage points going from pH 4.5 to 5.5, and then continued to decrease very gradually about 10 percentage points from pH 5.5 to 9.0. Figures 9-15 demonstrate these differences graphically.

The F-values, seen in Table 8, show significant differences for all of the fatty acids.

## Discussion

With changing pH, all seven of the major fatty acids demonstrated statistically significant differences. Again the most dramatic numerical differences were seen in the 18:1 and Δ19:0 fatty acids. Why the other five fatty acids show any differences at all is purely up to conjecture. If the proposal that the levels of 18:1 and Δ19:0 fatty acids correspond to "happiness" in this bacterium, then the results observed do make sense. Most rapid growth was observed at pH 6.0, very close to the high point of 18:1 at pH 6.5. Again the "happiness" point may not exactly correspond to most rapid growth. If the organism is "happiest" at pH 6.5, then the decrease in 18:1 as the pH is increased or decreased from that value would lend further support for the proposal that the high point of 18:1 content corresponds to optimum growth conditions for *S. lactis*.

Figure 9. Percentages of the 14:0 fatty acid from *S. lactis* grown at various pH's.

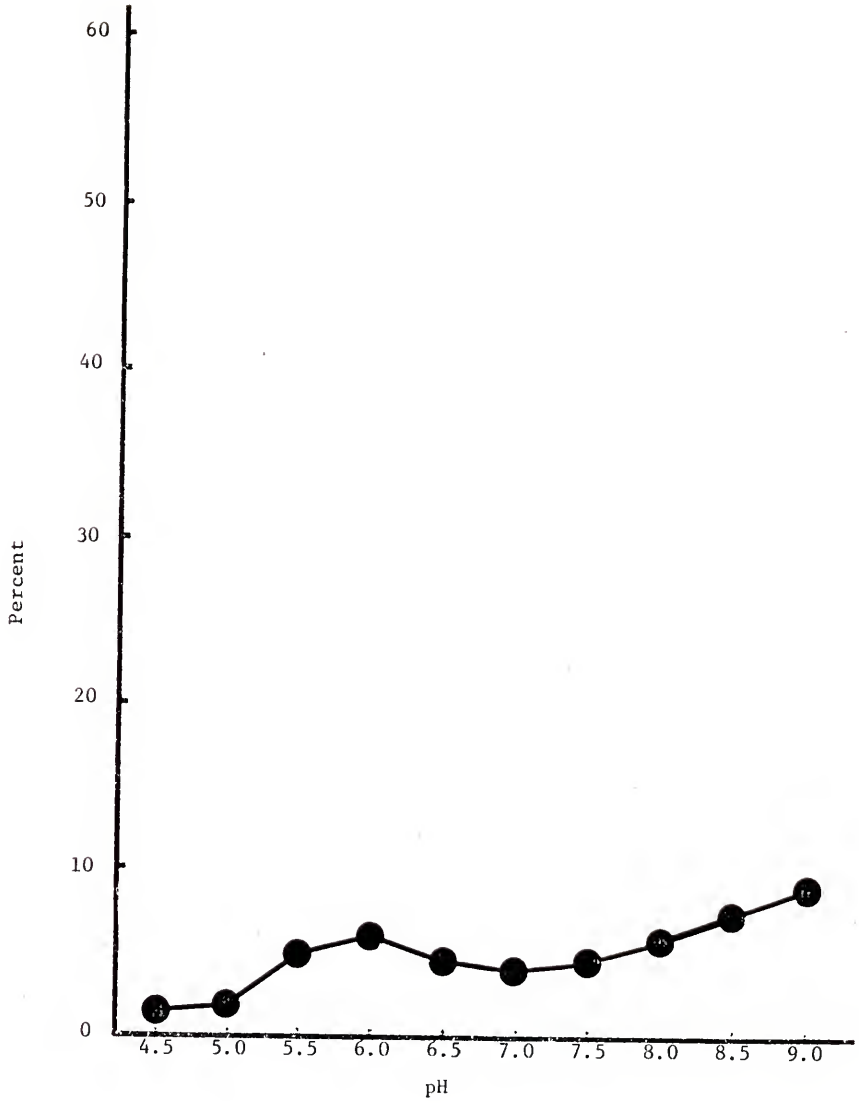

60
50
40
30
20
10
0
Percent
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
pH

Figure 10. Percentages of the 16:0 fatty acid from *S. lactis* grown at various pH's.

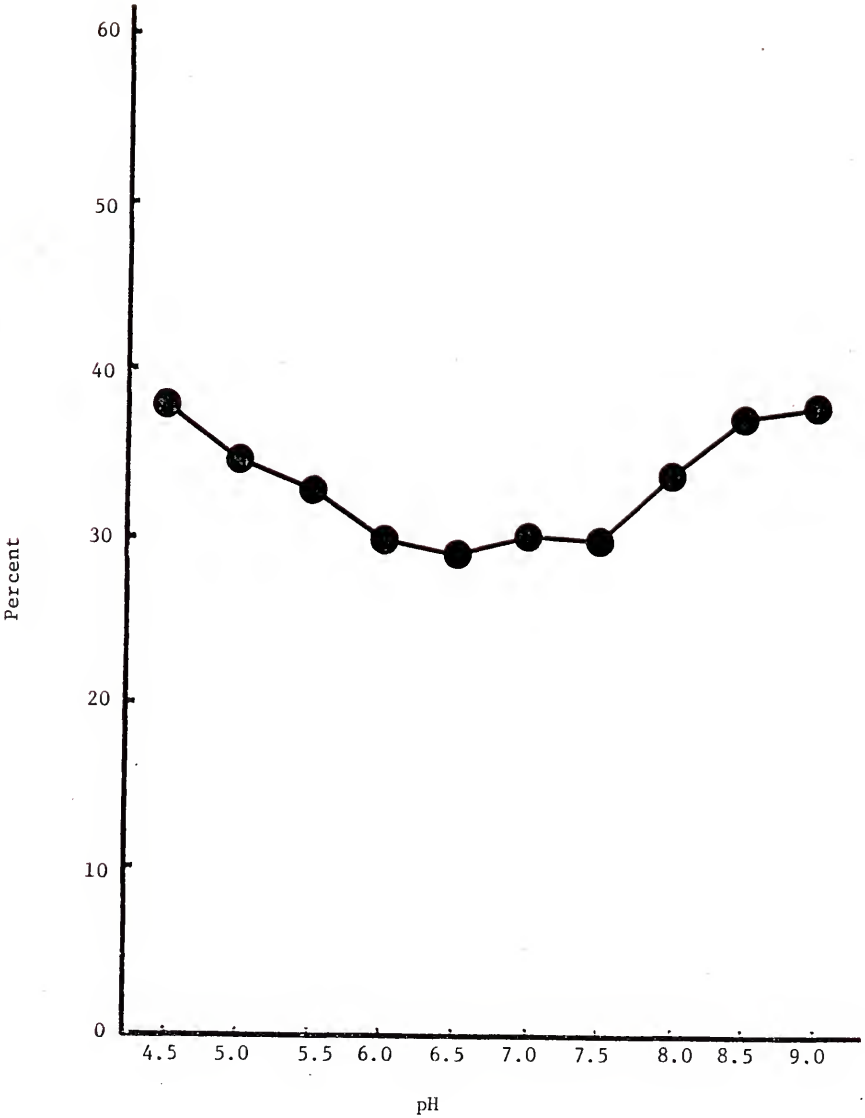

60
50
40
30
20
10
0
Percent
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
pH

Figure 11. Percentages of the 16:1 fatty acid from *S. lactis* grown at various pH's.

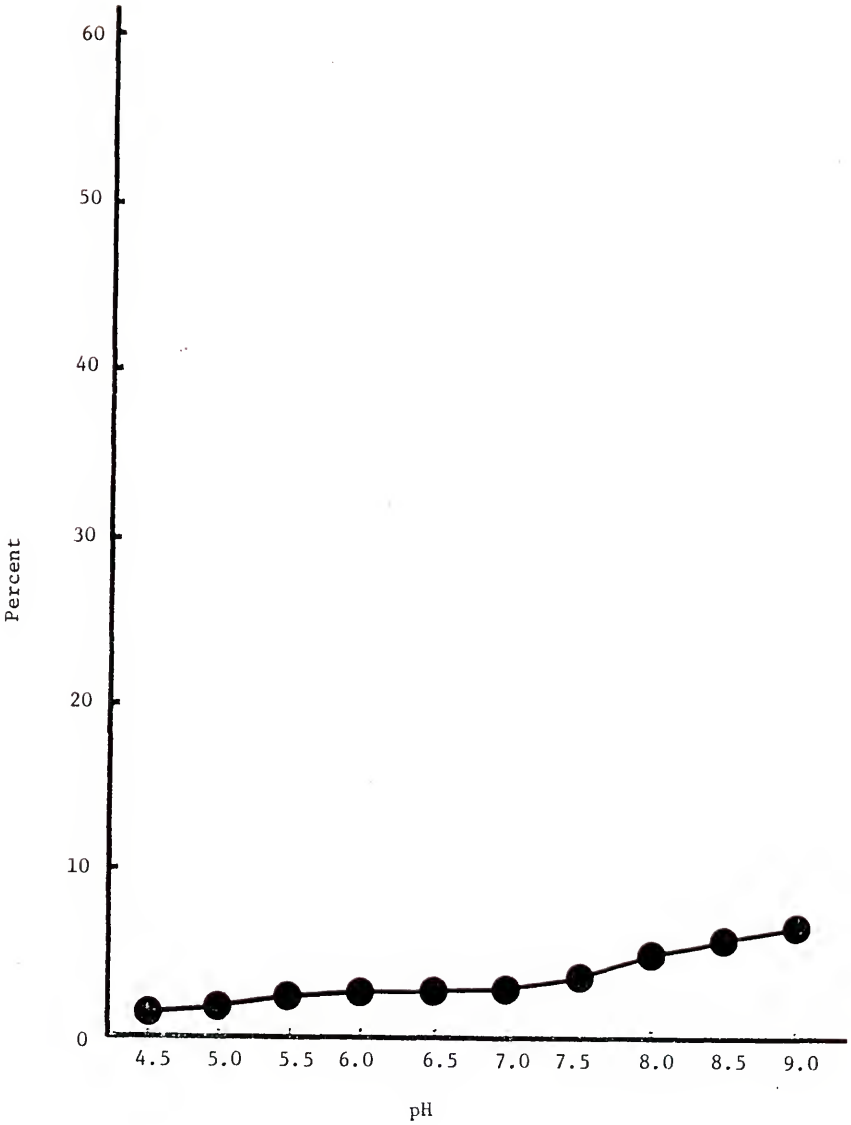

60
50
40
30
20
10
0
Percent
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
pH

Figure 12. Percentages of the 18:0 fatty acid from *S. lactis* grown at various pH's.

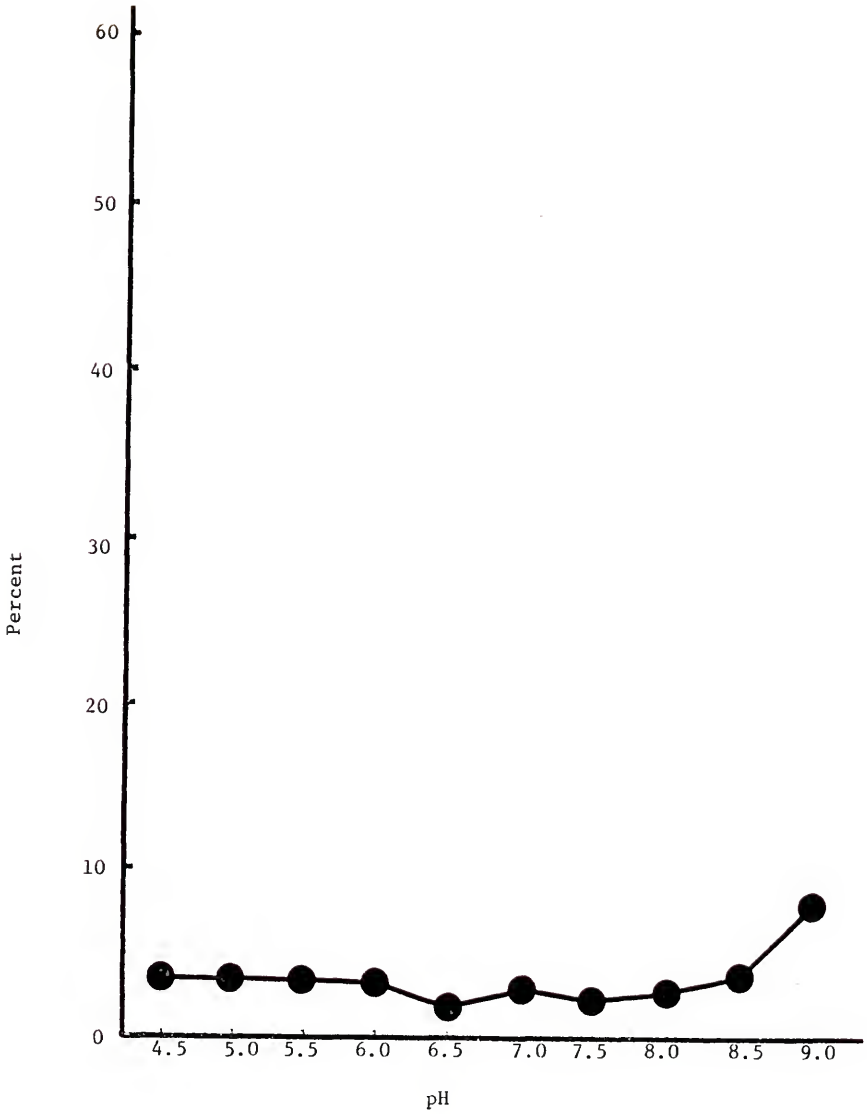

60
50
40
30
20
10
0
Percent
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
pH

Figure 13. Percentages of the 18:1 fatty acid from *S. lactis* grown at various pH's.

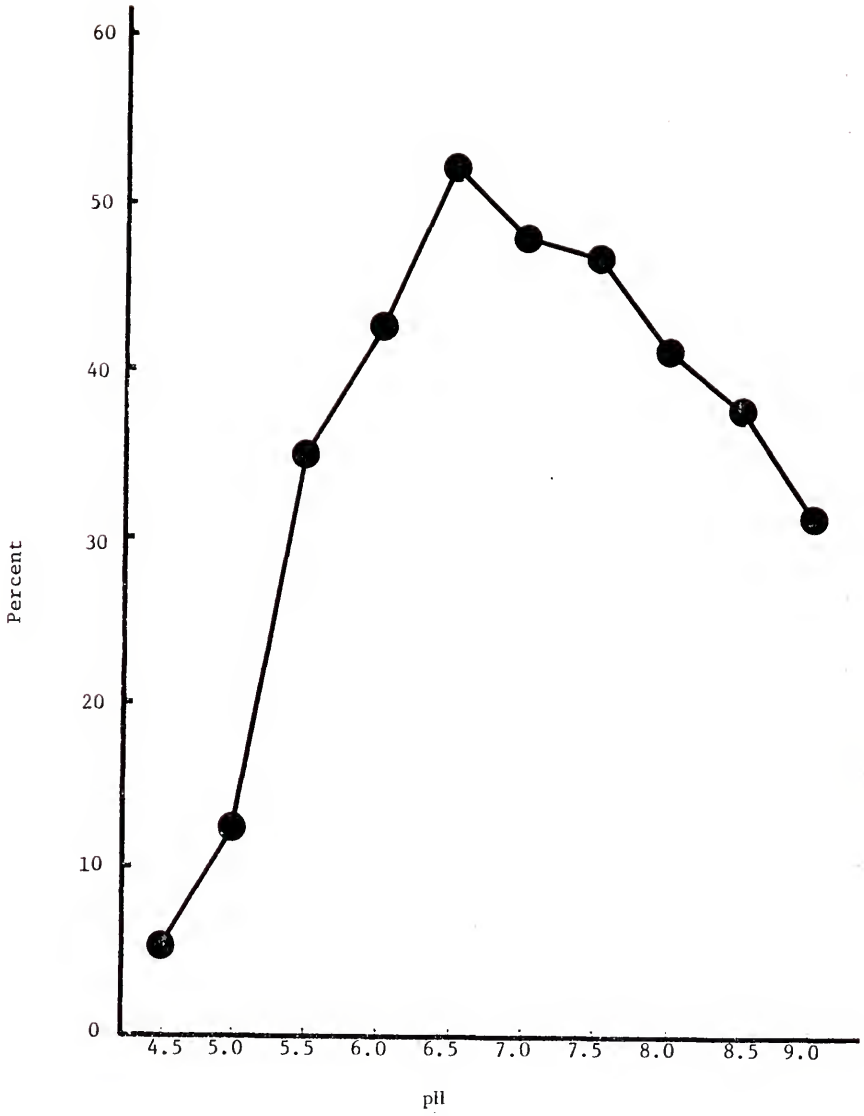

60
50
40
30
20
10
0
Percent
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
pH

Figure 14. Percentages of the Δ19:0 fatty acid from *S. lactis* grown at various pH's.

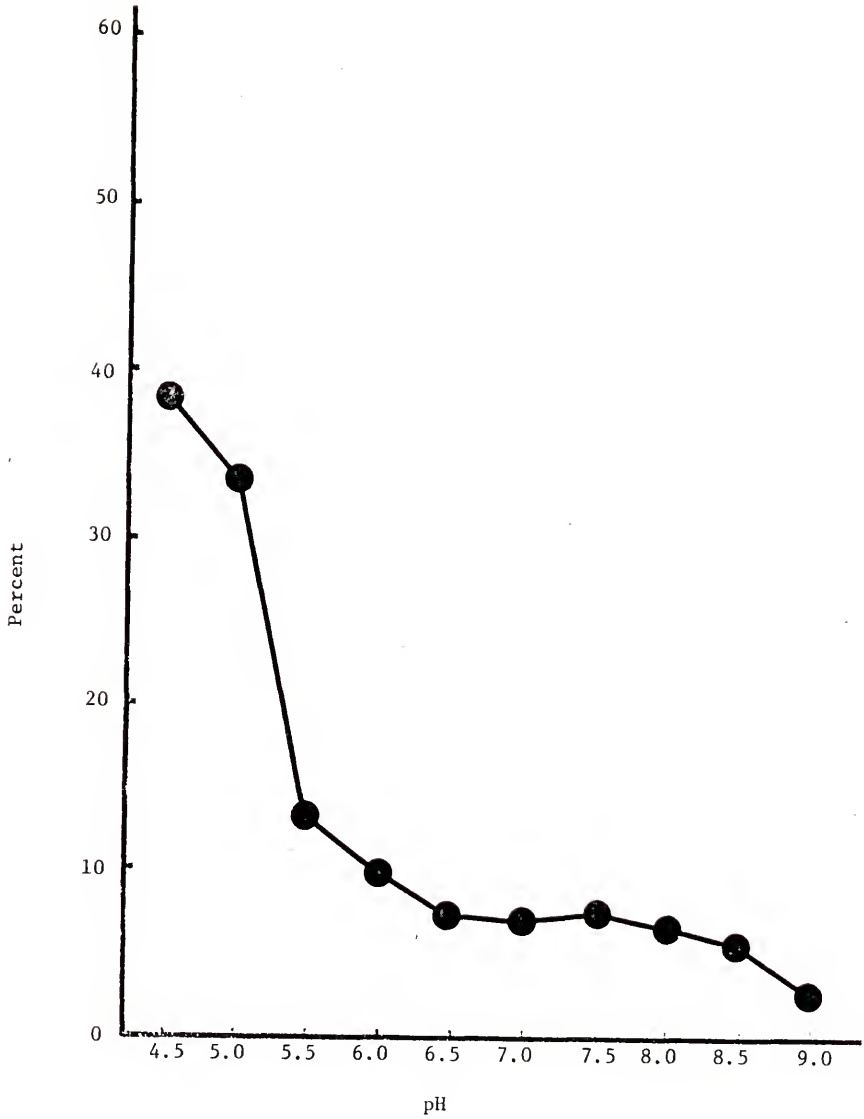

60
50
40
30
20
10
0
Percent
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
pH

Figure 15. Percentages of the 21:0 fatty acid from *S. lactis* grown at various pH's.

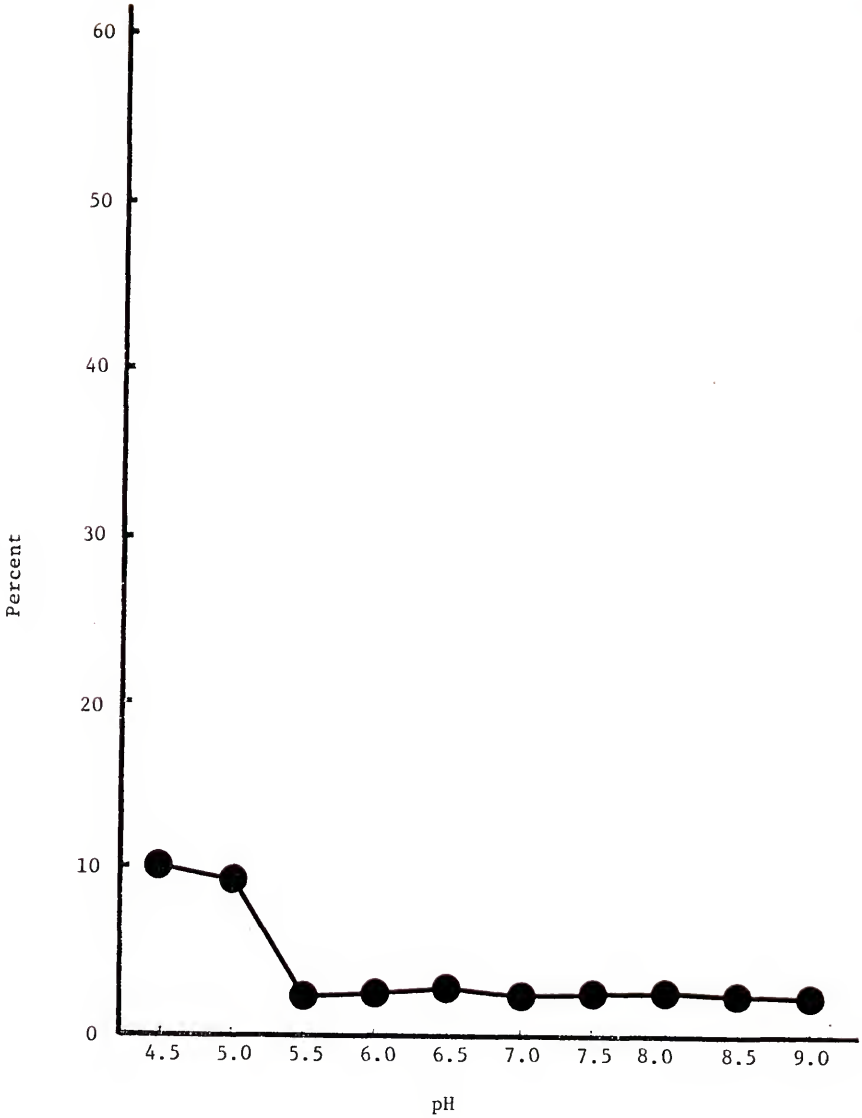

60
50
40
30
20
10
0
Percent
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
pH

Table 8. F-values and significance of differences for pH experiment.

| Fatty Acid | F-value[a] | Significance[b] |
|---|---|---|
| 14:0 | 9.60 | * |
| 16:0 | 6.98 | * |
| 16:1 | 29.87 | * |
| 18:0 | 4.79 | * |
| 18:1 | 152.32 | * |
| Δ19:0 | 75.55 | * |
| 21:0 | 23.39 | * |

[a] $F_{.05,9,20} = 2.39$

[b] * = significant

As for the Δ19:0 fatty acid, as the pH decreased from pH 6.5, the levels of Δ19:0 increased sharply, corresponding to an increase in adverse conditions. But above pH 6.5, the Δ19:0 level remained fairly constant and even decreased further at pH 9.0. Either the high pH is effecting the activity of the cyclopropane fatty acid synthetase, causing some sort of inhibition, or maybe the organism does not require the hypothetical protection afforded by the Δ19:0 fatty acid at the higher pH values.

# CHAPTER III
# EFFECTS OF GROWTH PHASE ON THE FATTY ACID COMPOSITION OF *S. lactis*

## Literature Review

Fatty acid composition in bacteria has long been known to vary with the phase of growth. In 1961 it was observed that *Lactobacillus arabinosus* harvested in stationary phase had higher proportions of lactobacillic acid and lower levels of unsaturated fatty acids than cells harvested during exponential phase (25). In cultures of *E. coli*, the accumulation of cyclopropane fatty acids at the expense of unsaturated fatty acids in stationary phase has also been reported (5,29). In *S. marcescens*, low amounts of cyclopropane fatty acids and high amounts of monoenoic fatty acids were present during early stages of growth, the latter being converted almost completely to cyclopropane fatty acids later in growth (26). The gram positive bacterium, *S. faecalis*, showed similar effects with cyclopropanes reaching a maximum level at the beginning of stationary phase (21,22). Analogous changes in the proportions of the 18:1 and Δ19:0 fatty acids of *Mycobacterium phei* have also been observed during the growth cycle (26).

Apparently most organisms studied with respect to growth phase have been those containing cyclopropane fatty acids, and in all cases cited the results are the same - cyclopropane fatty acids accumulate during stationary phase at the expense of their unsaturated fatty acid precursors.

## Additional Methods

Cells were grown in 1500 ml of medium in a 2.0 l jar. Sterile air was not pumped in since the 2.0 l jar had a very good seal as evidenced by culture purity at the end of the experiment. Samples of 150 ml were taken from the growth vessel at various phases of growth by way of an aspiration line from the growth vessel to a collection flask. Table 9 lists the times after inoculation that samples were collected as well as associated optical densities and the six phases of growth. This experiment was run in quadruplicate and an analysis of variance was applied to the data.

## Results

The percentage distributions of the fatty acids at each phase of growth are shown in Table 10. There was very little difference in relative fatty acid percentages between early and mid exponential phases. Both the iso 16:0 and 21:0 fatty acids remained at less than 1.0% of the total during these phases. More dramatic differences occurred when the cells reached late exponential and early stationary phases. The iso 16:0 content increased to 2.3% in late exponential phase, while the 21:0 content increased to 1.0% by early stationary phase. A decrease of nearly 12 percentage points was observed in the 18:1 content going from early exponential to early stationary phase, while the Δ19:0 fatty acid showed an increase of more than 6 percentage points over the same range. In late stationary phase, a further increase occurred in the iso 16:0, Δ19:0 and 21:0 fatty acids, with a further decrease occurring in the 18:1 fatty acids.

The 14:0, 16:0, 16:1 and 18:1 fatty acids showed very small differences throughout growth. The iso 16:0 and 21:0 fatty acids, although

Table 9. Sampling times and corresponding optical densities and phases of growth.

| Sample | Time after inoculation (hrs) | O.D. (540 nm) | Growth phase |
|---|---|---|---|
| 1 | 6.0 | 0.20 | Early exponential |
| 2 | 7.0 | 0.40 | Mid exponential |
| 3 | 8.5 | 0.80 | Late exponential |
| 4 | 11.0 | 1.30 | Early stationary |
| 5 | 16.0 | 1.30 | Mid stationary |
| 6 | 25.0 | 1.25 | Late stationary |

Table 10. Fatty acid percentages from *S. lactis* during various phases of growth.

| Fatty Acid | Exponential | | | Stationary | | |
|---|---|---|---|---|---|---|
| | Early | Mid | Late | Early | Mid | Late |
| 14:0 | 3.4 | 3.3 | 3.8 | 5.1 | 5.0 | 4.9 |
| iso 16:0 | <1.0 | <1.0 | 2.3 | 2.4 | 3.3 | 3.5 |
| 16:0 | 30.2 | 29.6 | 29.3 | 31.1 | 29.7 | 28.9 |
| 16:1 | 2.1 | 2.0 | 2.1 | 2.6 | 2.5 | 2.6 |
| 18:0 | 3.5 | 2.5 | 3.0 | 2.9 | 3.5 | 3.8 |
| 18:1 | 57.4 | 58.0 | 53.0 | 45.6 | 42.0 | 39.7 |
| Δ19:0 | 2.1 | 3.0 | 5.1 | 8.4 | 11.7 | 12.1 |
| 21:0 | <1.0 | <1.0 | <1.0 | 1.0 | 1.5 | 1.6 |

increasing from trace amounts in the initial phases to a few percent in the later phase, still only showed relatively minor differences. Only the 18:1 and Δ19:0 fatty acids showed relatively large differences. These differences are graphically represented in Figures 16-23.

The F-values, listed in Table 11, indicate that the 14:0, iso 16:0, 18:1, Δ19:0 and 21:0 fatty acids demonstrate significant differences while the 16:0, 16:1 and 18:0 fatty acids do not.

## Discussion

Five of eight major fatty acids showed statistically significant differences in this experiment. The predominant differences in the 18:1 and Δ19:0 contents are not unexpected. As cells proceed from exponential to stationary phase, decreases in unsaturates with increases in cyclopropanes have been reported to occur in other bacteria such as *E. coli*, *S. marcescens* and *S. faecalis* (21,22,29,31).

Summing up the percentages of the 18:1 and Δ19:0 fatty acids at each phase of growth gives the values listed in Table 12. Again these sums represent the relative amounts of 18:1 fatty acid incorporated into membrane lipids during growth. The sums decrease approximately 9 percentage points from mid exponential to late stationary phase. Since Δ19:0 shows an increase of 10 percentage points and the 18:1 fatty acid a decrease of 18 percentage points, it becomes apparent that nearly half of the decrease in the 18:1 content is due to conversion to the Δ19:0 fatty acid and the other half is due to decreasing amounts of the 18:1 fatty acid being incorporated into membrane lipid. Therefore, it seems that *S. lactis* adjusts its membrane fatty acid composition, in response

Figure 16. Percentages of the 14:0 fatty acid from *S. lactis* at various times during growth.

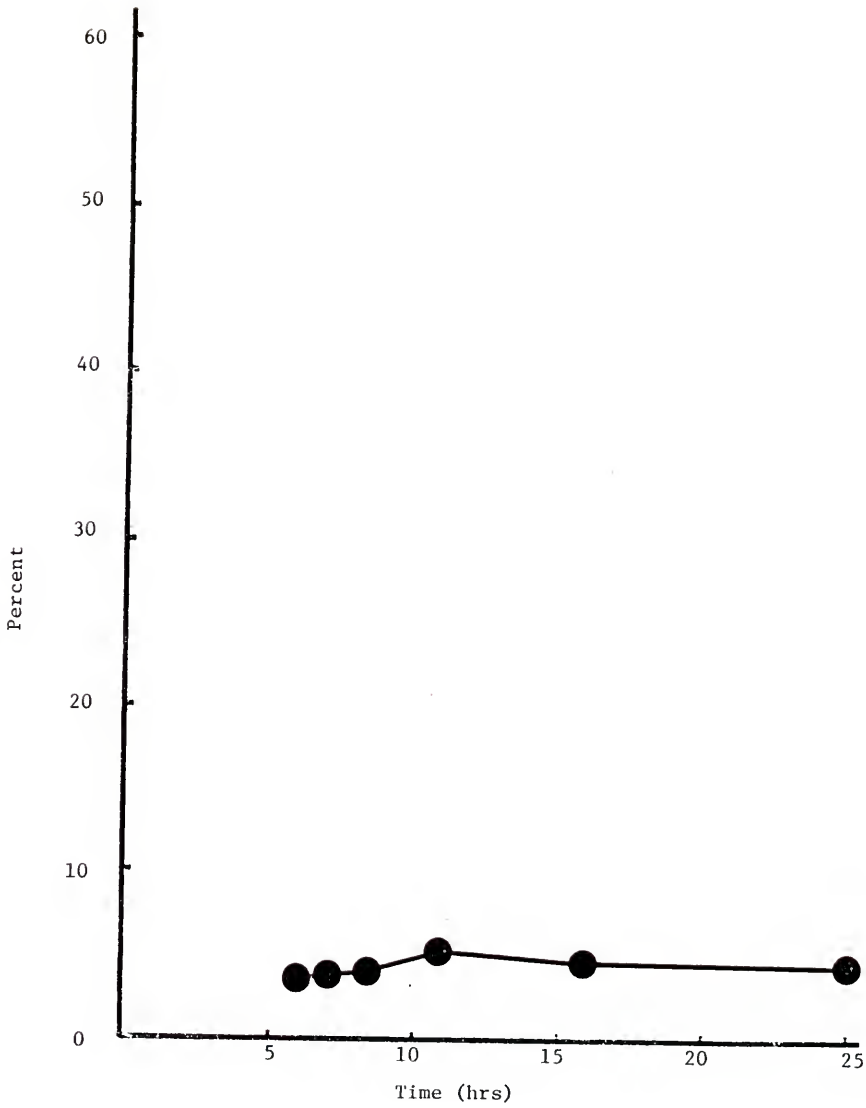

60
50
40
30
20
10
0
Percent
5
10
15
20
25
Time (hrs)

Figure 17. Percentages of the iso 16:0 fatty acid from *S. lactis* at various times during growth.

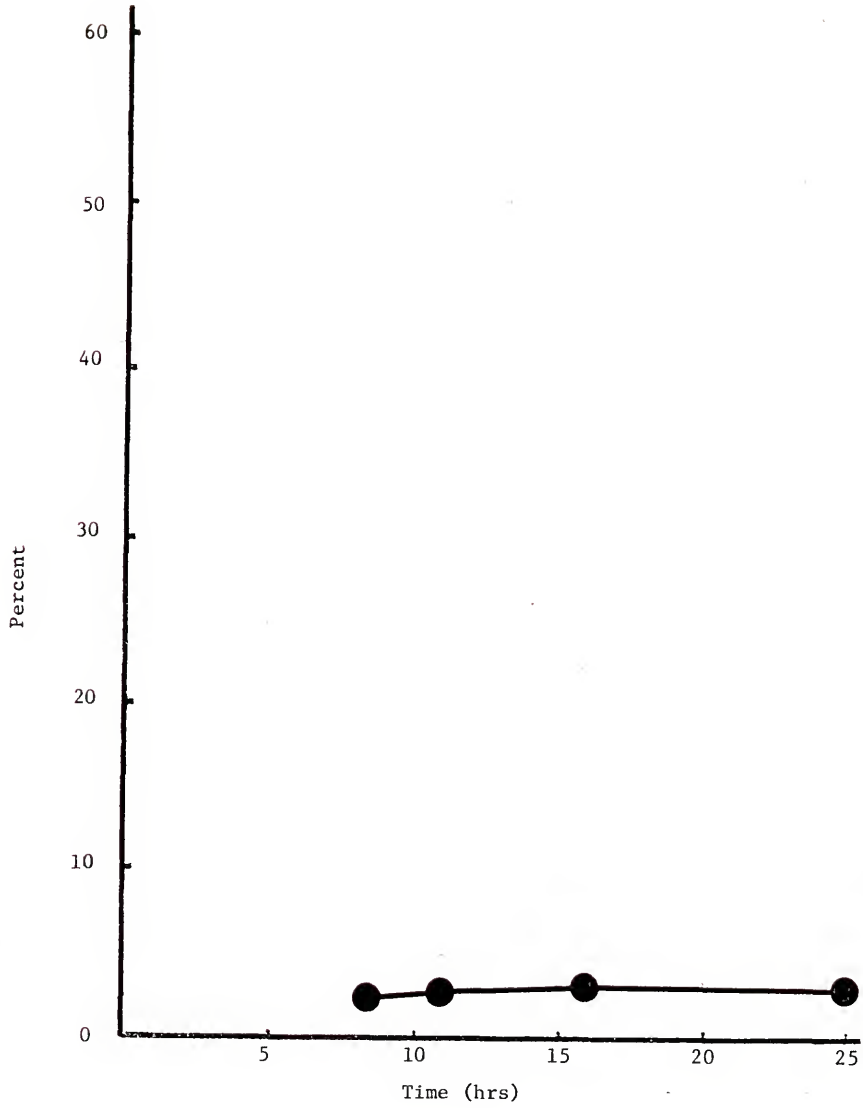

60
50
40
30
20
10
0
Percent
5
10
15
20
25
Time (hrs)

Figure 18. Percentages of the 16:0 fatty acid from *S. lactis* at various times during growth.

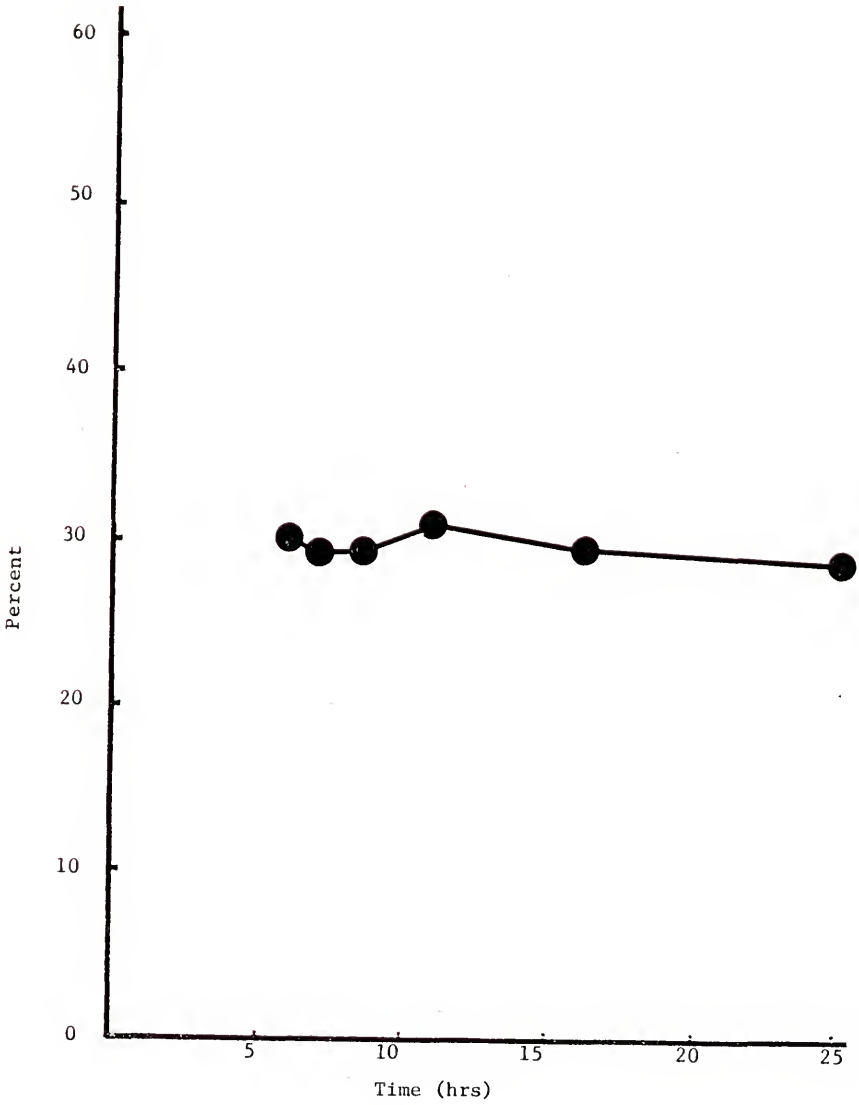

60
50
40
30
20
10
0
Percent
5
10
15
20
25
Time (hrs)

Figure 19. Percentages of the 16:1 fatty acid from *S. lactis* at various times during growth.

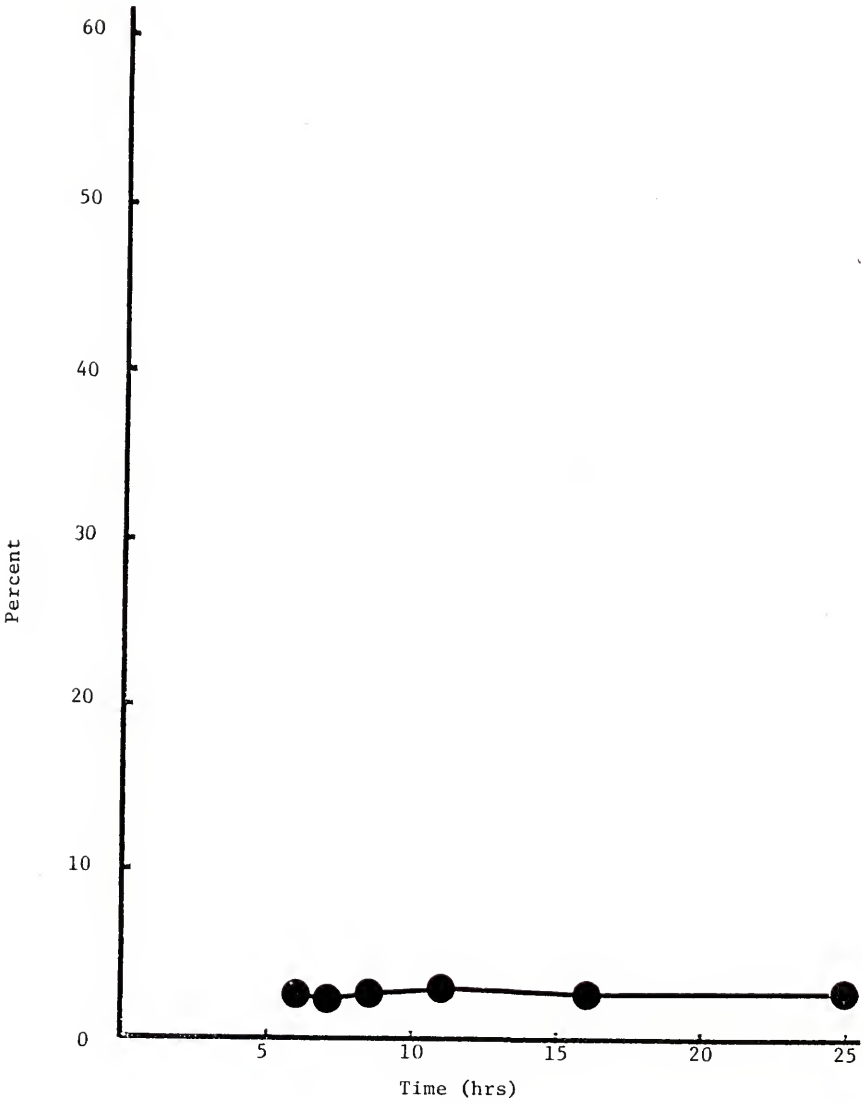

60
50
40
30
20
10
0
Percent
5
10
15
20
25
Time (hrs)

Figure 20. Percentages of the 18:0 fatty acid from *S. lactis* at various times during growth.

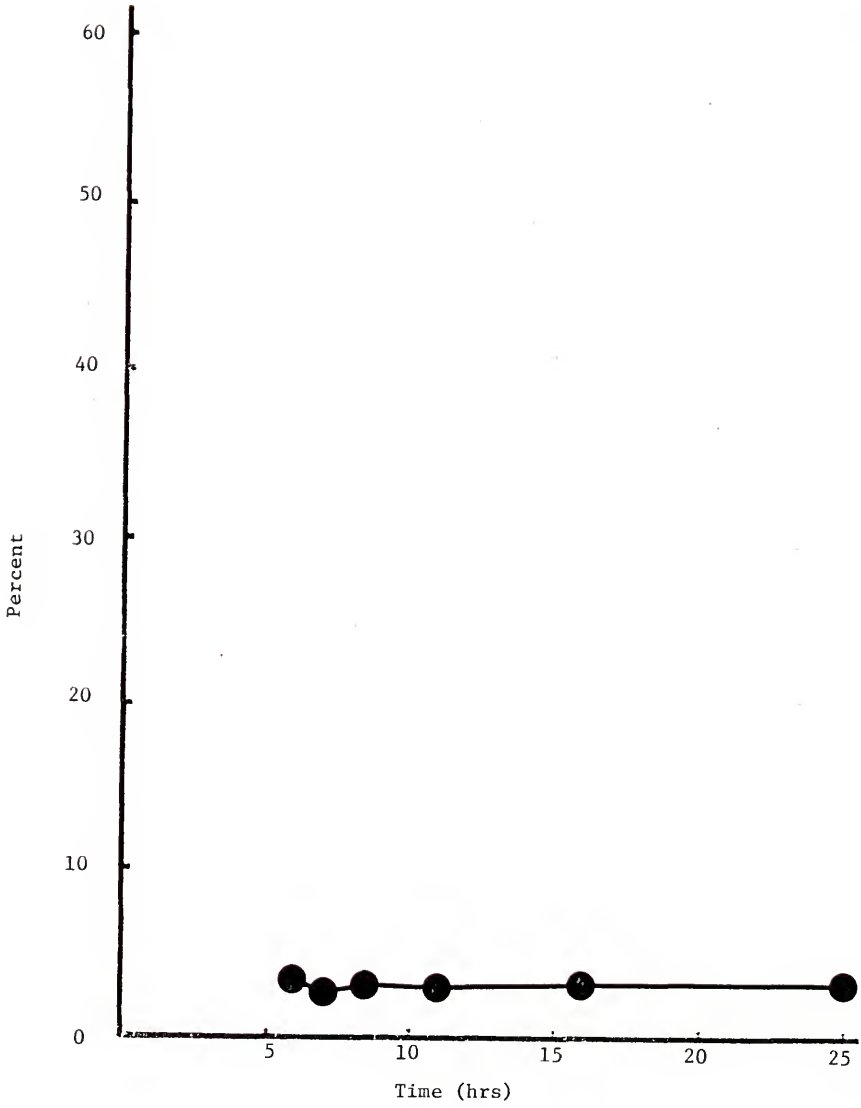

60
50
40
30
20
10
0
Percent
5
10
15
20
25
Time (hrs)

Figure 21. Percentages of the 18:1 fatty acid from *S. lactis* at various times during growth.

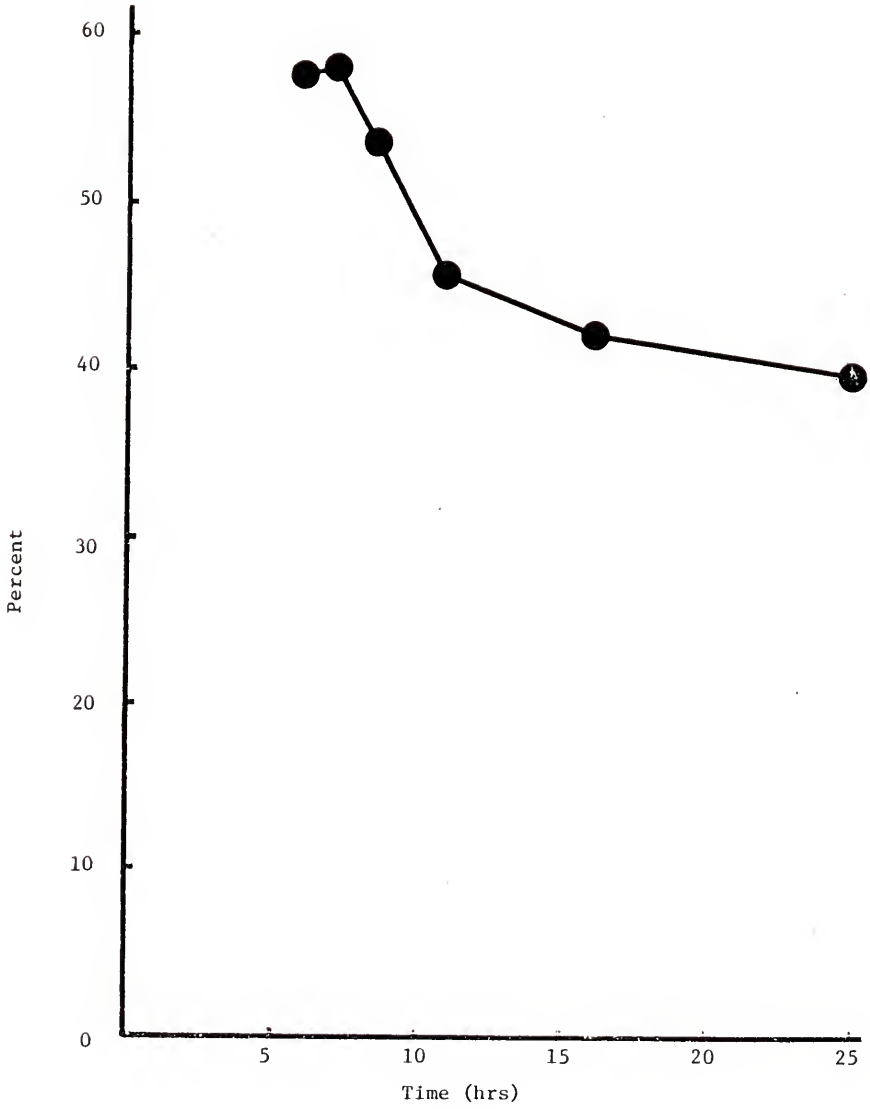

60
50
40
30
20
10
0
Percent
5
10
15
20
25
Time (hrs)

Figure 22. Percentages of the Δ19:0 fatty acid from *S. lactis* at various times during growth.

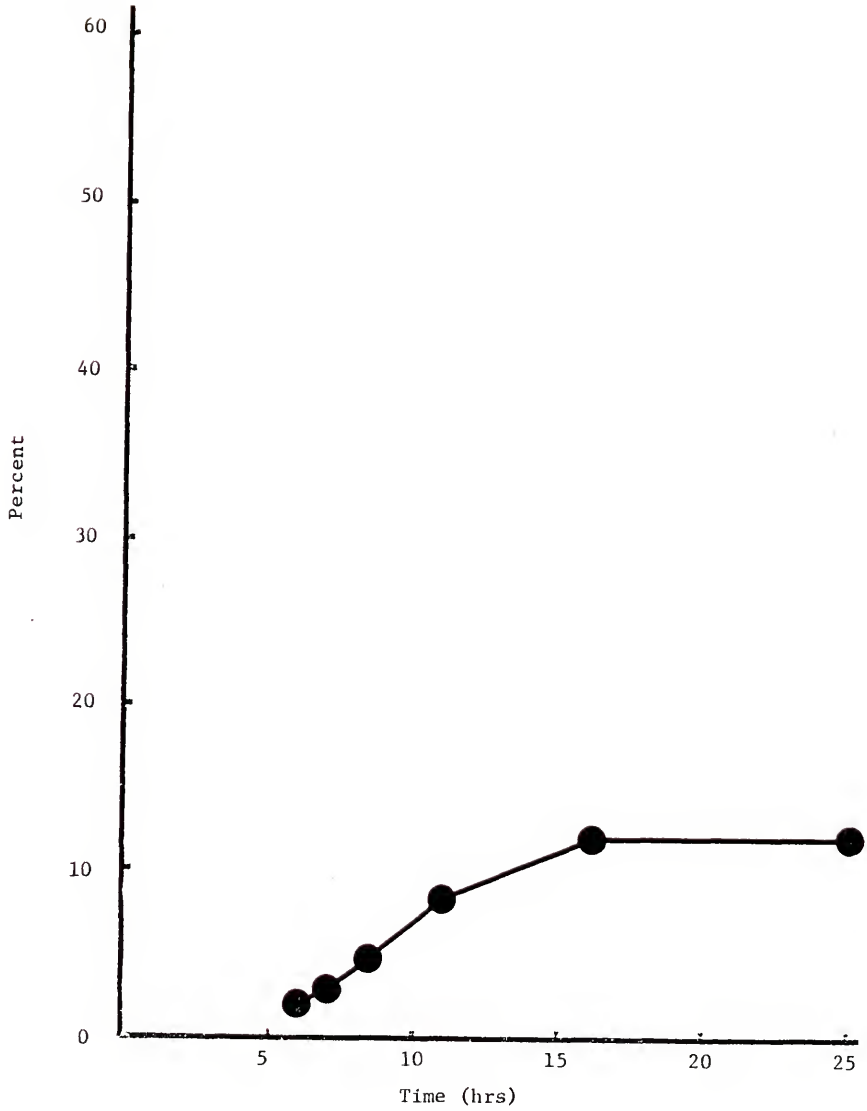

60
50
40
30
20
10
0
Percent
5
10
15
20
25
Time (hrs)

Figure 23. Percentages of the 21:0 fatty acid from *S. lactis* at various times during growth.

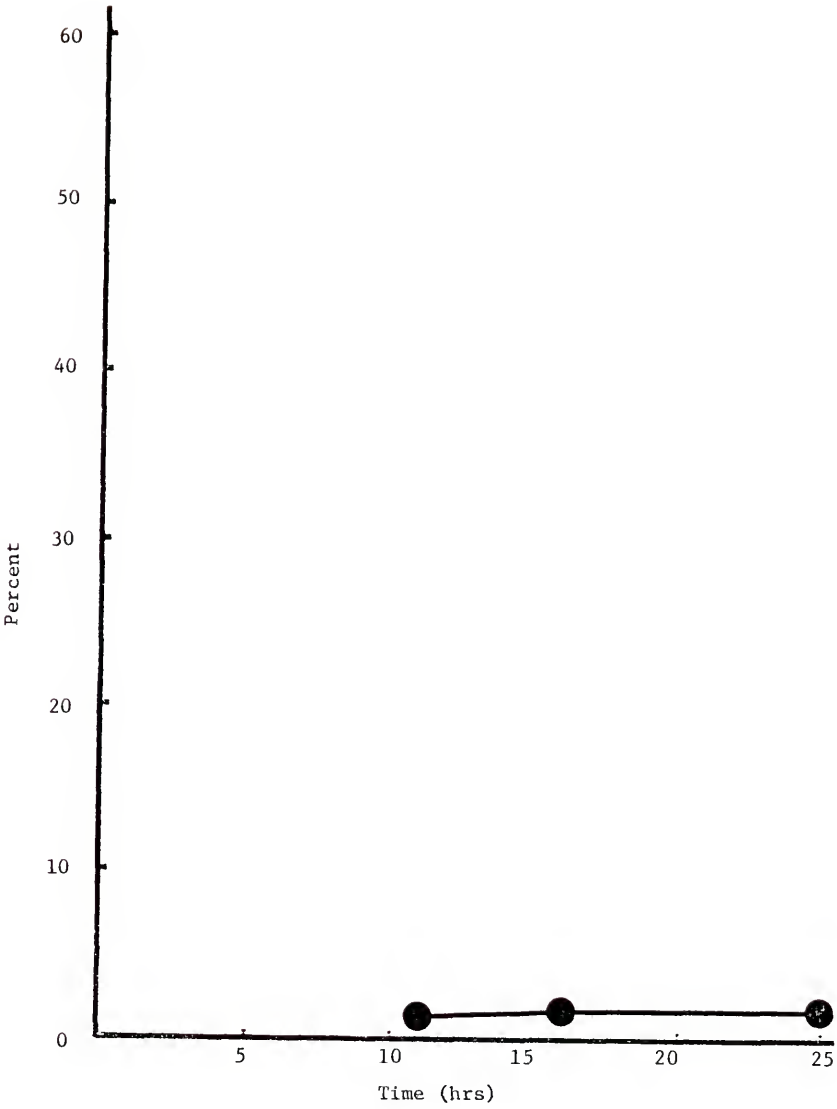

60
50
40
30
20
10
0
Percent
5
10
15
20
25
Time (hrs)

Table 11. F-values and significance of differences for growth phase experiment.

| Fatty Acid | F-value[a] | Significance |
|---|---|---|
| 14:0 | 50.17 | * |
| iso 16:0 | 6.04 | * |
| 16:0 | 1.61 | NS |
| 16:1 | 2.65 | NS |
| 18:0 | 0.53 | NS |
| 18:1 | 132.96 | * |
| Δ19:0 | 77.44 | * |
| 21:0 | 15.10 | * |

[a] $F_{.05,5,18} = 2.77$

[b] * = significant; NS = not significant

Table 12. Sums of 18:1 and Δ19:0 fatty acid percentages at each phase of growth.

| Phase of growth | 18:1 | Δ19:0 | 18:1 + Δ19:0 |
|---|---|---|---|
| Early exponential | 57.4 | 2.1 | 59.5 |
| Mid exponential | 58.0 | 3.0 | 61.0 |
| Late exponential | 53.0 | 5.1 | 58.1 |
| Early stationary | 45.6 | 8.4 | 54.0 |
| Mid stationary | 42.0 | 11.7 | 53.7 |
| Late stationary | 39.7 | 12.1 | 51.8 |

to changes in growth phase, primarily in two ways - it decreases its overall incorporation of 18:1 fatty acid and increases it content of Δ19:0 fatty acid.

# CHAPTER IV
# EFFECTS OF VARIOUS CARBOHYDRATE SOURCES ON THE FATTY ACID COMPOSITION OF *S. lactis*

## Literature Review

Reports on the influence of carbohydrate source on bacterial fatty acid composition are scarce. In 1974, Drucker, Griffith and Melville (15) compared fatty acid profiles of two strains of *S. mutans* and one strain of *S. faecalis* when grown on 1.0% glucose and 3.0% sucrose. The iso 14:0 was 35 percentage points higher in one strain of *S. mutans* grown on the sucrose compared to growth on the glucose. *S. faecalis* showed a similar result, but only 7 percentage points. The same strain of *S. mutans* showed lesser amounts of 16:0 and 16:1 fatty acids when grown on sucrose, 20.0% and 4.0% respectively. The same organism also demonstrated 12 percentage points less 18:1 fatty acid on sucrose compared to glucose. *S. faecalis* had 5 percentage points lower 18:0 content and 10 percentage points lower 18:1 content on sucrose compared to glucose. Carbohydrate source does have an effect on fatty acid composition, but it depends on the organism as well as the carbohydrate.

## Additional Methods

Organisms were grown in 0.056 M concentrations of the following carbohydrates: trehalose, dextrose, lactose, glucosamine, cellobiose, fructose, galactose, mannose, salicin, maltose, xylose, ribose, and mannitol.

A doubling time for growth on each carbohydrate was determined from growth curves. This experiment was run in triplicate.

## Results

Doubling times for growth on the different carbohydrates are listed in Table 13. Growth was most rapid on trehalose, while mannitol accommodated the slowest growth.

The fatty acid percentages for growth on each carbohydrate are listed in Table 14. The 18:0 fatty acid content remained fairly constant. The 14:0 and 16:1 fatty acids showed relatively small differences (5 percentage points at most). These same fatty acids, however, did reach their highest levels in cells grown on carbohydrates generating the slowest growth. The largest difference in the 16:0 fatty acid content was 9 percentage points with no obvious relationship to growth rate. The 21:0 fatty acid remained constant, also with one relatively high percentage value when the organism was grown on fructose.

Again the 18:1 and Δ19:0 fatty acids demonstrated the only dramatic differences. A difference as large as 30 percentage points was seen between growth on trehalose and xylose in the 18:1 fatty acid with the least amounts again somewhat related to slow growth. The Δ19:0 fatty acid demonstrated its largest difference of about 16 percentage points between growth on fructose and xylose with the highest amounts related to slow growth. Figures 24-30 graphically demonstrate these results.

Table 13. Doubling times of *S. lactis* grown on various carbohydrate sources.

| Carbohydrate | Doubling time (min.) |
|---|---|
| Trehalose | 42 |
| Dextrose | 55 |
| Lactose | 65 |
| Glucosamine | 67 |
| Cellobiose | 72 |
| Fructose | 75 |
| Galactose | 90 |
| Mannose | 90 |
| Salicin | 90 |
| Maltose | 116 |
| Xylose | 183 |
| Ribose | 342 |
| Mannitol | 391 |

Table 14. Fatty acid percentages from *S. lactis* grown on various carbohydrate sources

| Fatty acid | Carbohydrate | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1[a] | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| 14:0 | 5.4 | 3.7 | 3.9 | 4.0 | 5.8 | 4.1 | 4.0 | 3.7 | 5.3 | 6.6 | 7.3 | 7.4 | 10.0 |
| 16:0 | 26.9 | 33.9 | 29.7 | 30.5 | 27.8 | 29.9 | 27.2 | 28.8 | 25.0 | 27.3 | 32.9 | 32.3 | 32.9 |
| 16:1 | 3.2 | 2.1 | 3.7 | 2.4 | 3.1 | 3.2 | 2.7 | 2.4 | 2.7 | 3.6 | 5.1 | 4.4 | 7.6 |
| 18:0 | 1.5 | 2.7 | 1.7 | 1.6 | 1.9 | 1.7 | 1.6 | 2.0 | 1.1 | 1.5 | 3.1 | 2.2 | 3.2 |
| 18:1 | 54.8 | 46.8 | 48.7 | 51.6 | 53.5 | 45.0 | 52.4 | 53.1 | 54.8 | 42.6 | 25.7 | 38.6 | 28.6 |
| Δ19:0 | 4.9 | 5.8 | 8.4 | 4.4 | 4.1 | 2.5 | 8.3 | 2.9 | 3.3 | 11.8 | 19.6 | 8.3 | 10.6 |
| 21:0 | 1.7 | 2.5 | 1.7 | 2.3 | 2.1 | 6.8 | 1.6 | 3.4 | 2.4 | 2.7 | 4.1 | 2.4 | 2.8 |

[a] 1 = trehalose; 2 = dextrose; 3 = lactose; 4 = glucosamine; 5 = cellobiose; 6 = fructose; 7 = galactose; 8 = mannose; 9 = salicin; 10 = maltose; 11 = xylose; 12 = ribose; 13 = mannitol.

Figure 24. Percentages of the 14:0 fatty acid from *S. lactis* grown on various carbohydrate sources.

[a] 1 = trehalose; 2 = dextrose; 3 = lactose; 4 = glucosamine; 5 = cellobiose; 6 = fructose; 7 = galactose; 8 = mannose; 9 = salicin; 10 = maltose; 11 = xylose; 12 = ribose; 13 = mannitol.

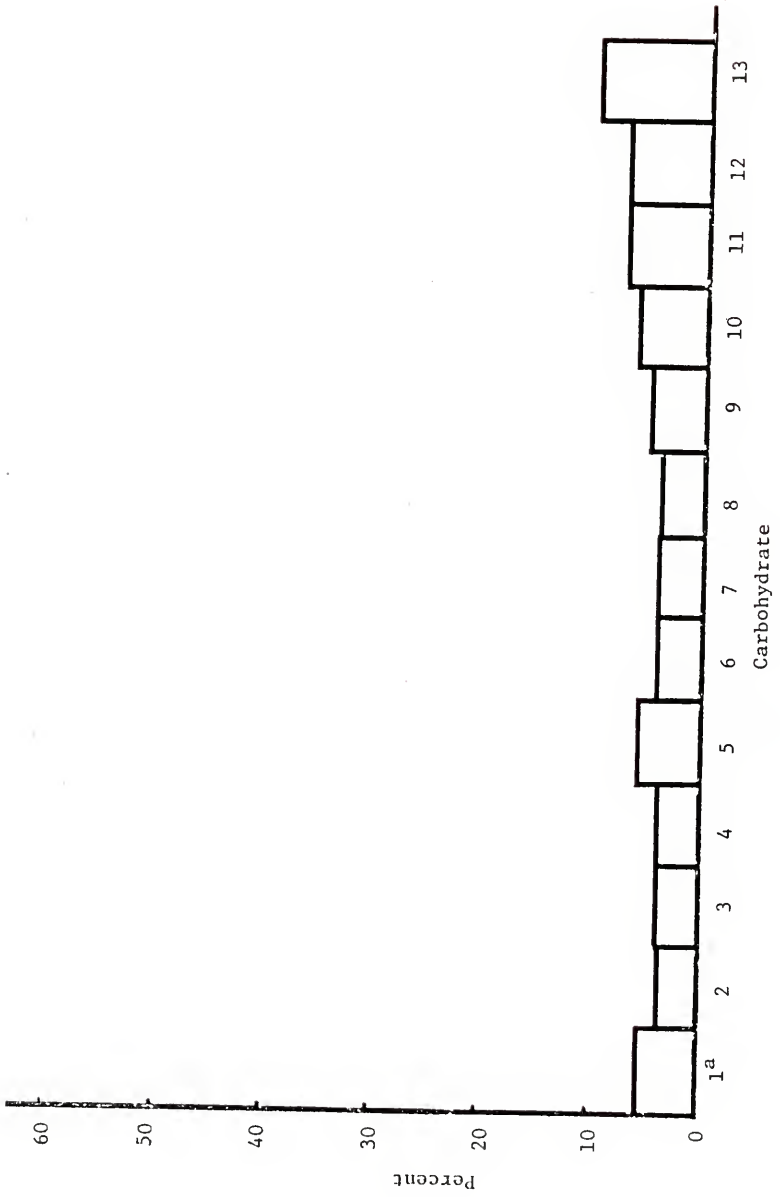

Percent
60
50
40
30
20
10
0
1a
2
3
4
5
6
7
8
9
10
11
12
13
Carbohydrate

Figure 25. Percentages of the 16:0 fatty acid from *S. lactis* grown on various carbohydrate sources.

[a] 1 = trehalose; 2 = dextrose; 3 = lactose; 4 = glucosamine; 5 = cellobiose; 6 = fructose; 7 = galactose; 8 = mannose; 9 = salicin; 10 = maltose; 11 = xylose; 12 = ribose; 13 = mannitol.

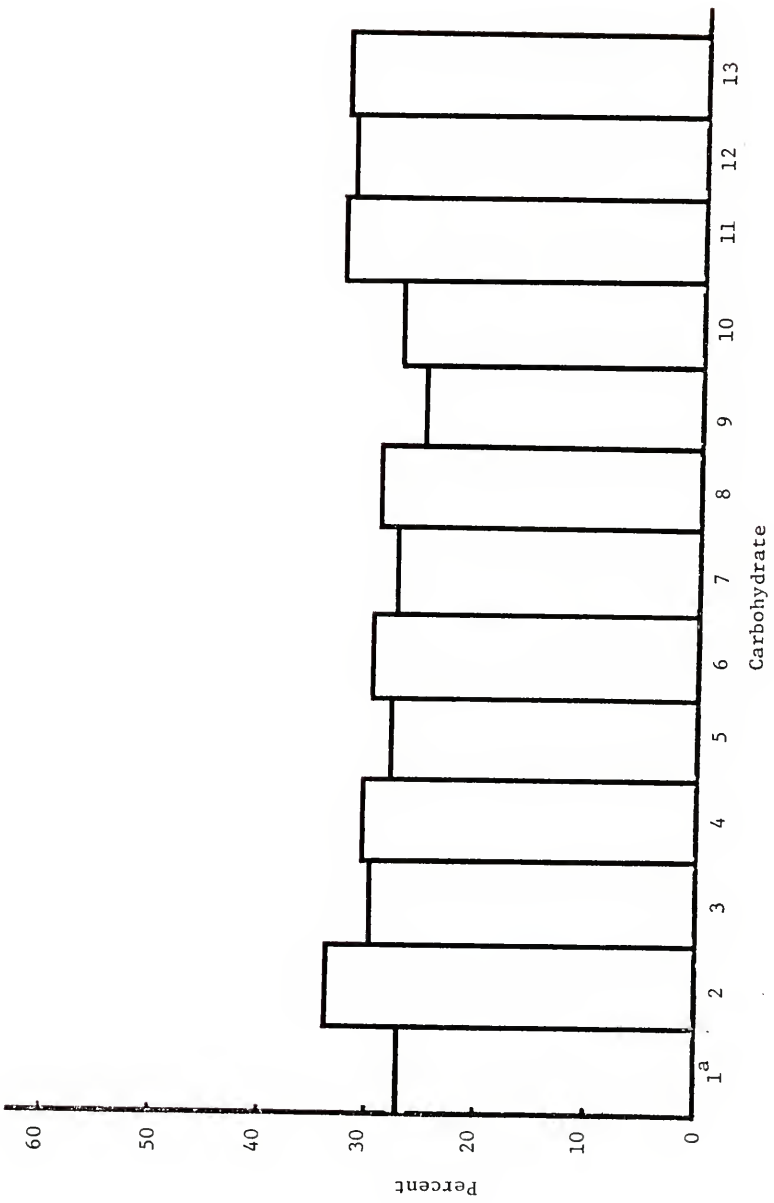

Percent
60
50
40
30
20
10
0
1[a]
2
3
4
5
6
7
8
9
10
11
12
13
Carbohydrate

Figure 26. Percentages of the 16:1 fatty acid from *S. lactis* grown on various carbohydrate sources.

[a] 1 = trehalose; 2 = dextrose; 3 = lactose; 4 = glucosamine; 5 = cellobiose; 6 = fructose; 7 = galactose; 8 = mannose; 9 = salicin; 10 = maltose; 11 = xylose; 12 = ribose; 13 = mannitol.

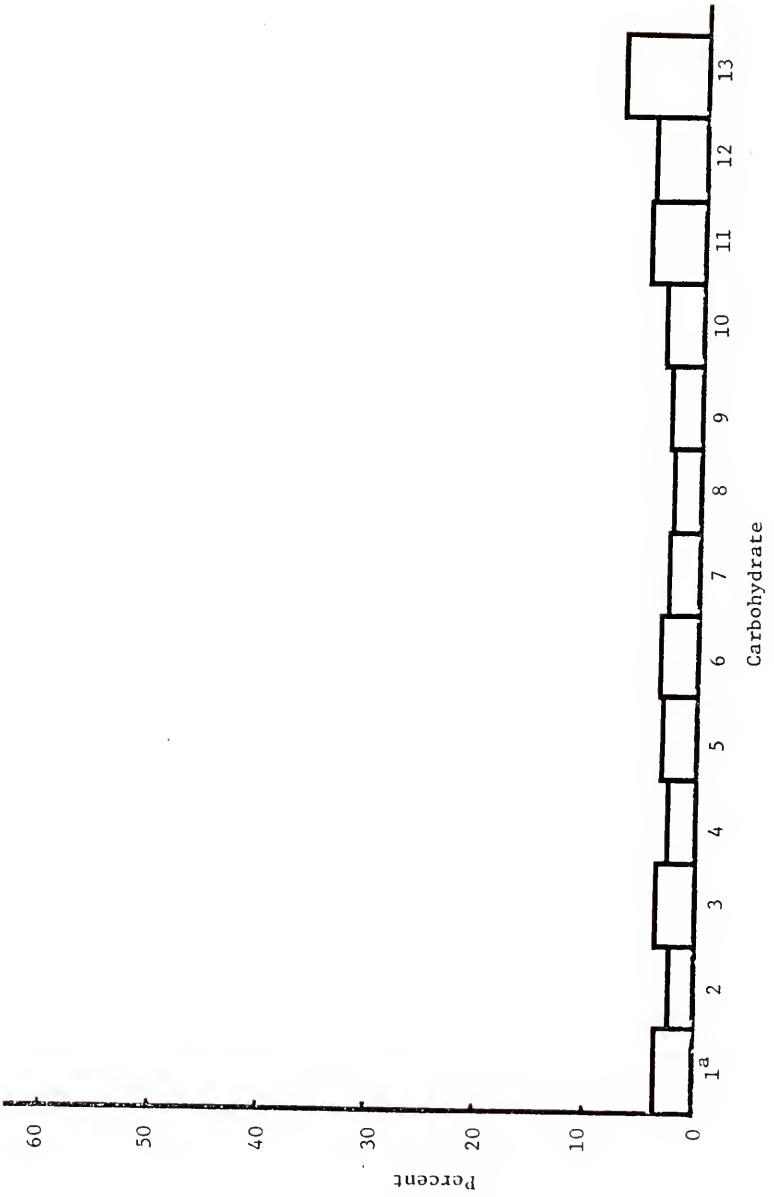

Percent
60
50
40
30
20
10
0
1a
2
3
4
5
6
7
8
9
10
11
12
13
Carbohydrate

Figure 27. Percentages of the 18:0 fatty acid from *S. lactis* grown on various carbohydrate sources.

[a] 1 = trehalose; 2 = dextrose; 3 = lactose; 4 = glucosamine; 5 = cellobiose; 6 = fructose; 7 = galactose; 8 = mannose; 9 = salicin; 10 = maltose; 11 = xylose; 12 = ribose; 13 = mannitol.

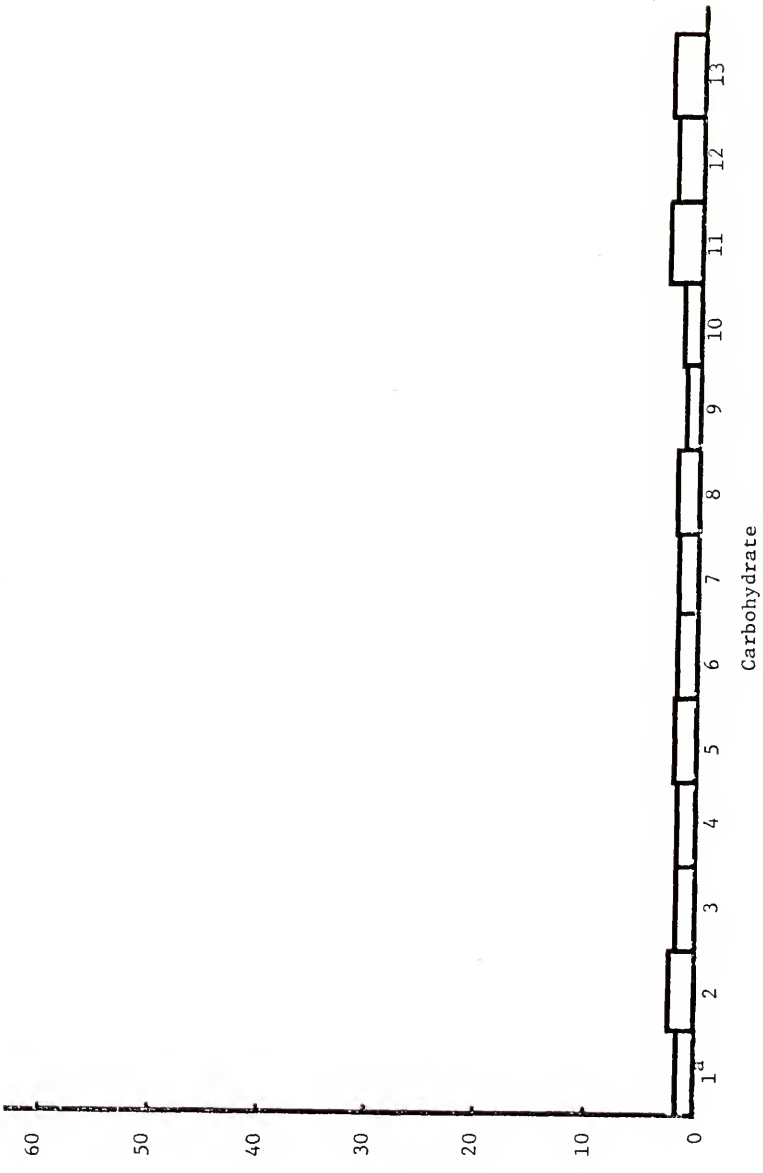

Percent
60
50
40
30
20
10
0
1d
2
3
4
5
6
7
8
9
10
11
12
13
Carbohydrate

Figure 28. Percentages of the 18:1 fatty acid from *S. lactis* grown on various carbohydrate sources.

[a] 1 = trehalose; 2 = dextrose; 3 = lactose; 4 = glucosamine; 5 = cellobiose; 6 = fructose; 7 = galactose; 8 = mannose; 9 = salicin; 10 = maltose; 11 = xylose; 12 = ribose; 13 = mannitol.

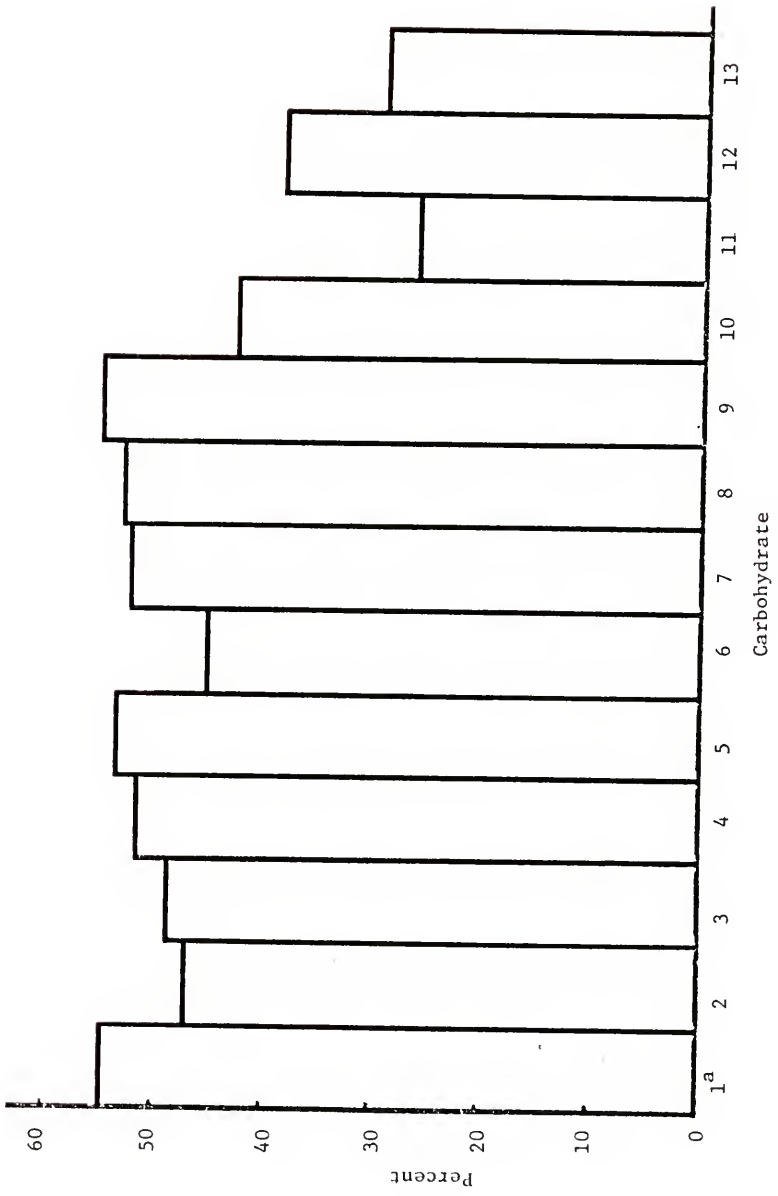

Percent
0
10
20
30
40
50
60
1[a]
2
3
4
5
6
7
8
9
10
11
12
13
Carbohydrate

Figure 29. Percentages of the Δ19:0 fatty acid from *S. lactis* grown on various carbohydrate sources.

[a] 1 = trehalose; 2 = dextrose; 3 = lactose; 4 = glucosamine; 5 = cellobiose; 6 = fructose; 7 = galactose; 8 = mannose; 9 = salicin; 10 = maltose; 11 = xylose; 12 = ribose; 13 = mannitol.

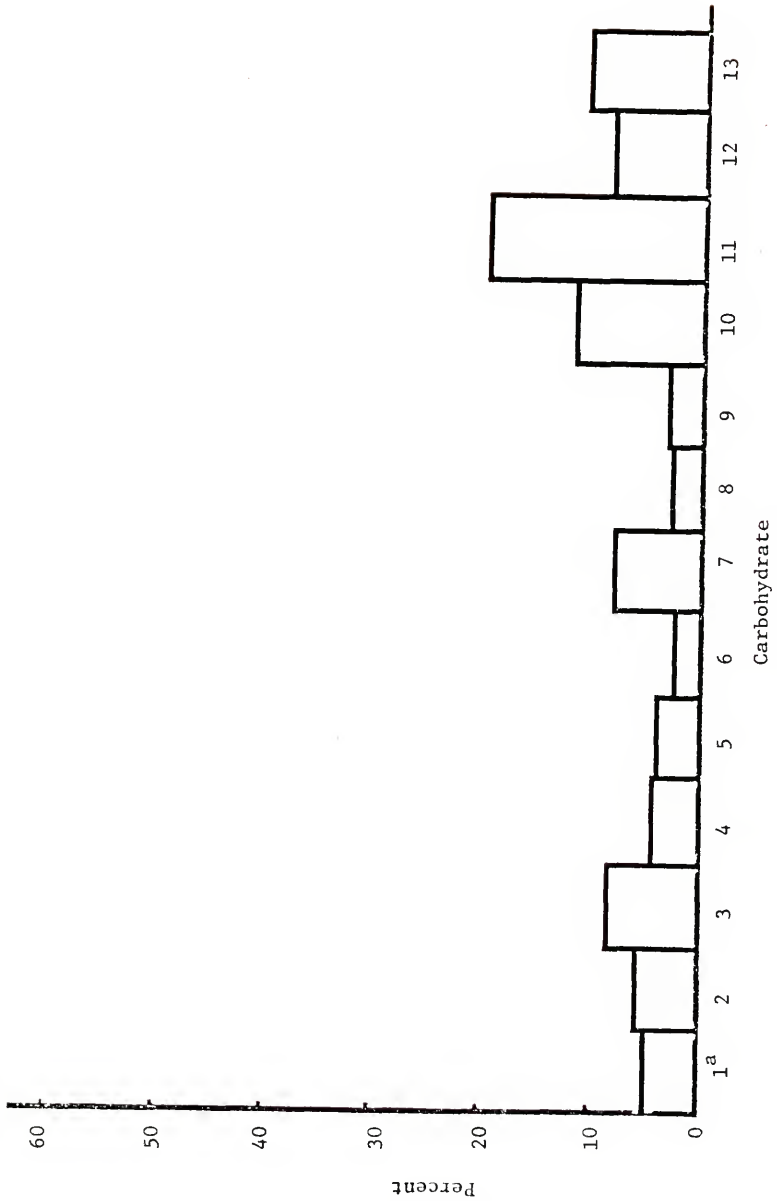
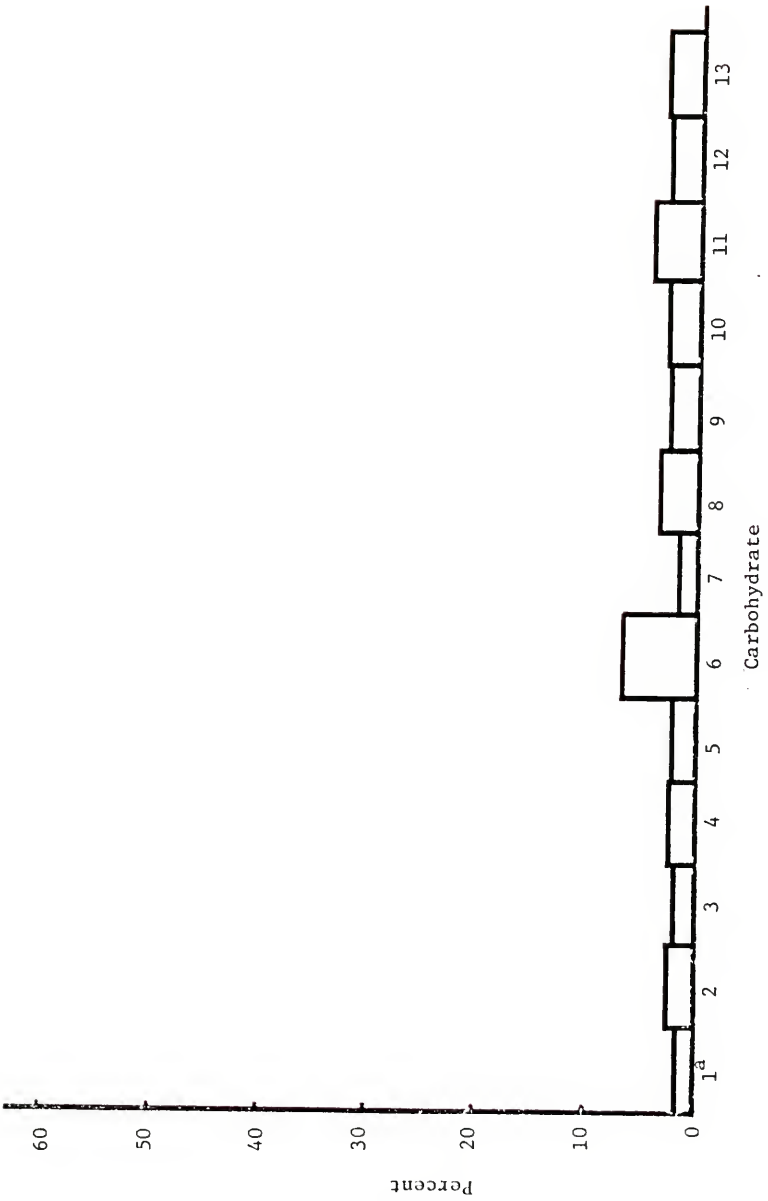

Percent
60
50
40
30
20
10
0
1[a]
2
3
4
5
6
7
8
9
10
11
12
13
Carbohydrate

Figure 30. Percentages of the 21:0 fatty acid from *S. lactis* grown on various carbohydrate sources.

[a] 1 trehalose; 2 = dextrose; 3 = lactose; 4 = glucosamine; 5 = cellobiose; 6 = fructose; 7 = galactose; 8 = mannose; 9 = salicin; 10 = maltose; 11 = xylose; 12 = ribose; 13 = mannitol.


Percent
60
50
40
30
20
10
0
1[a]
2
3
4
5
6
7
8
9
10
11
12
13
Carbohydrate

## Discussion

The results from this experiment may again lend support to the hypothesis that the most rapid growth rate is not necessarily coincident with a "happy" organism. Even though the lowest levels of the 18:1 fatty acid and the highest levels of the Δ19:0 fatty acid are found associated with carbohydrates effecting slower rates of growth, a direct correlation does not seem to be the case. For example, growth on salicin is relatively slow, yet its 18:1 content is the highest and its Δ19:0 content is very low.

# CHAPTER V
# EFFECTS OF CITRATE ON THE FATTY ACID COMPOSITION OF *S. lactis*

## Literature Review

In eucaryotic systems, enzymes concerned with citrate metabolism play an important role in fatty acid biosynthesis. Investigators have shown that citrate was incorporated more rapidly into fatty acids than acetate *in vitro* using yeast enzymes (39).

Effects of citrate on fatty acid composition in a bacterium, *E. coli*, have also been investigated. In a medium containing 0.1 g/l of trisodium citrate, the percentage of the Δ17:0 fatty acid was below 6.0% when the cell density was less than 1 g/l. As the cell density increased above 1 g/l, the percentage of the Δ17:0 fatty acid increased and exceeded 12.0%. There was an accompanying decrease in the 16:1 content. Cells grown in the absence of citrate had a higher percentage of Δ17:0 fatty acid than did cells grown in its presence, provided that the cell concentration exceeded 1 g/l. One consequence of adding citrate to the medium is chelation of $Mg^{++}$, a cofactor required for the formation of cyclopropane fatty acid (29).

## Additional Methods

Cells were grown in the presence of the following: no citrate, 0.0075 mM diammonium citrate, 0.075 mM diammonium citrate, 0.75 mM diammonium citrate, 7.5 mM diammonium citrate, 75.0 mM diammonium citrate, and 7.5 mM diammonium phosphate. The media containing no citrate and 7.5 mM diammonium phosphate acted as controls.

Doubling times were determined from growth curves, and an analysis of variance was applied to the data. This experiment was run in triplicate.

## Results

Growth with various concentrations of citrate resulted in the doubling times listed in Table 15. All of the doubling times were nearly the same except for the one associated with the highest concentration of citrate which was 10 minutes slower.

Table 16 lists the fatty acid percentages at each concentration of citrate. The 14:0, 16:0, 16:1, 18:0 and 21:0 fatty acids remained fairly constant in content. The 18:1 fatty acid also remained fairly constant except at the highest citrate concentration where it showed a decrease of about 5 percentage points. The Δ19:0 fatty acid was even more constant with only a slight rise in content of about 3 percentage points at the highest citrate level. These results are represented graphically in Figures 31-37.

Table 17 shows F-values and significance of differences. When the five concentrations of citrate plus no-citrate are compared, the 14:0, 18:0, 18:1, Δ19:0 and 21:0 fatty acids show significant differences. When no-citrate is compared to diammonium phosphate, only the 18:0 fatty acid demonstrates significance of differences. This is also true when 7.5 mM diammonium citrate is compared to 7.5 mM diammonium phosphate.

Table 15. Doubling times of *S. lactis* grown with various concentrations of citrate.

| Diammonium citrate | Doubling time (min.) |
|---|---|
| none | 76 |
| 0.0075 mM | 75 |
| 0.075 mM | 74 |
| 0.75 mM | 77 |
| 7.5 mM | 77 |
| 75.0 mM | 87 |
| **Diammonium phosphate** | **Doubling time (min.)** |
| 7.5 mM | 76 |

Table 16. Fatty acid percentages from *S. lactis* grown with various concentrations of citrate.

| Fatty acid | Citrate | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1[a] | 2 | 3 | 4 | 5 | 6 | 7 |
| 14:0 | 3.7 | 3.8 | 3.9 | 3.8 | 3.7 | 3.3 | 4.0 |
| 16:0 | 30.9 | 32.0 | 32.2 | 32.5 | 32.7 | 32.1 | 33.6 |
| 16:1 | 2.3 | 2.2 | 2.2 | 2.3 | 2.4 | 2.9 | 2.4 |
| 18:0 | 1.8 | 1.6 | 1.4 | 2.0 | 2.0 | 2.8 | 1.5 |
| 18:1 | 51.6 | 52.3 | 51.1 | 51.9 | 49.2 | 44.3 | 49.6 |
| Δ19:0 | 6.4 | 5.5 | 5.3 | 4.4 | 6.0 | 7.8 | 5.4 |
| 21:0 | 2.3 | 1.8 | 1.9 | 1.8 | 2.3 | 3.5 | 2.8 |

[a] 1 = no citrate added; 2 = 0.0075 mM diammonium citrate; 3 = 0.075 mM diammonium citrate; 4 = 0.75 mM diammonium citrate; 5 = 7.5 mM diammonium citrate; 6 = 75.0 mM diammonium citrate; 7 = 7.5 mM diammonium phosphate.

Figure 31. Percentages of the 14:0 fatty acid from *S. lactis* grown with various concentrations of citrate.

[a] 1 = no citrate; 2 = 0.0075 mM diammonium citrate; 3 = 0.075 mM diammonium citrate; 4 = 0.75 mM diammonium citrate; 5 = 7.5 mM diammonium citrate; 6 = 75.0 mM diammonium citrate; 7 = 7.5 mM diammonium phosphate.

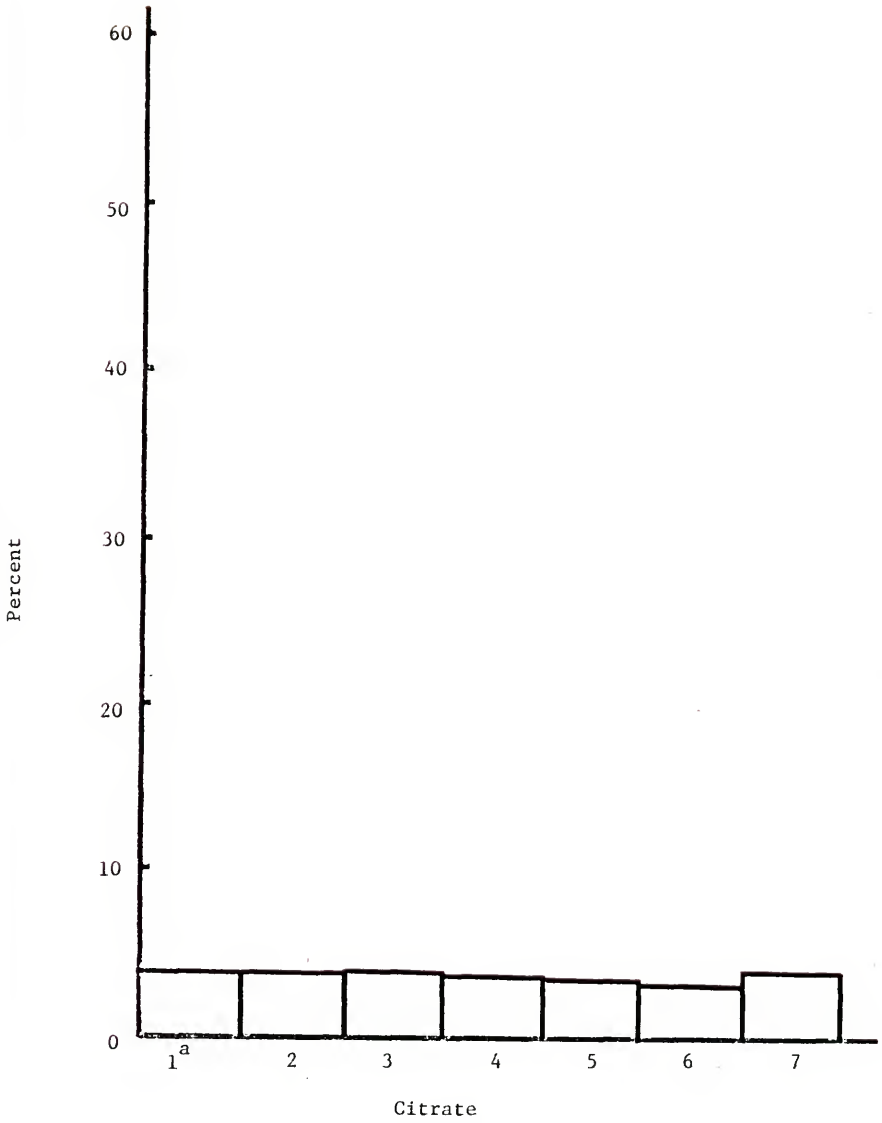

60
50
40
30
20
10
0
Percent
1[a]
2
3
4
5
6
7
Citrate

Figure 32. Percentages of the 16:0 fatty acid from *S. lactis* grown with various concentrations of citrate.

[a] 1 = no citrate; 2 = 0.0075 mM diammonium citrate; 3 = 0.075 mM diammonium citrate; 4 = 0.75 mM diammonium citrate; 5 = 7.5 mM diammonium citrate; 6 = 75.0 mM diammonium citrate; 7 = 7.5 mM diammonium phosphate.

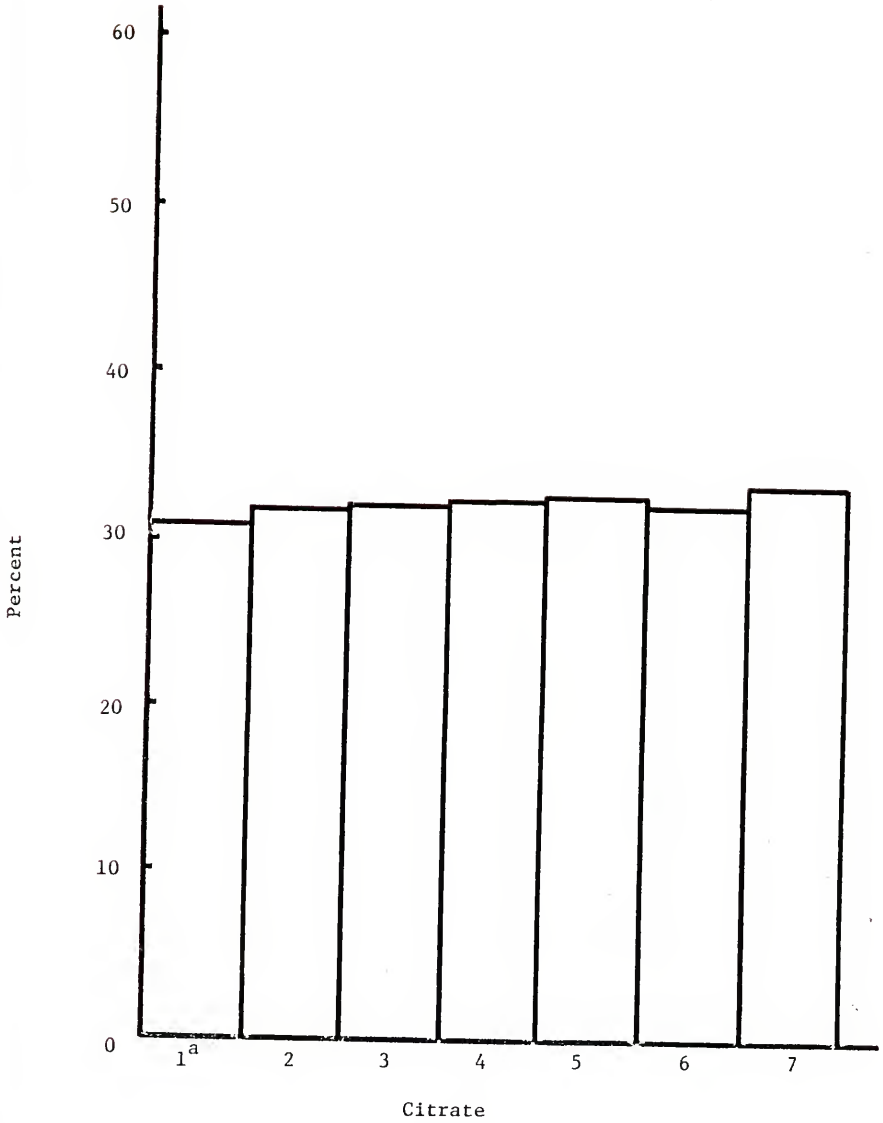

60
50
40
30
20
10
0
Percent
1[a]
2
3
4
5
6
7
Citrate

Figure 33. Percentages of the 16:1 fatty acid from *S. lactis* grown with various concentrations of citrate.

[a] 1 = no citrate; 2 = 0.0075 mM diammonium citrate; 3 = 0.075 mM diammonium citrate; 4 = 0.75 mM diammonium citrate; 5 = 7.5 mM diammonium citrate; 6 = 75.0 mM diammonium citrate; 7 = 7.5 mM diammonium phosphate.

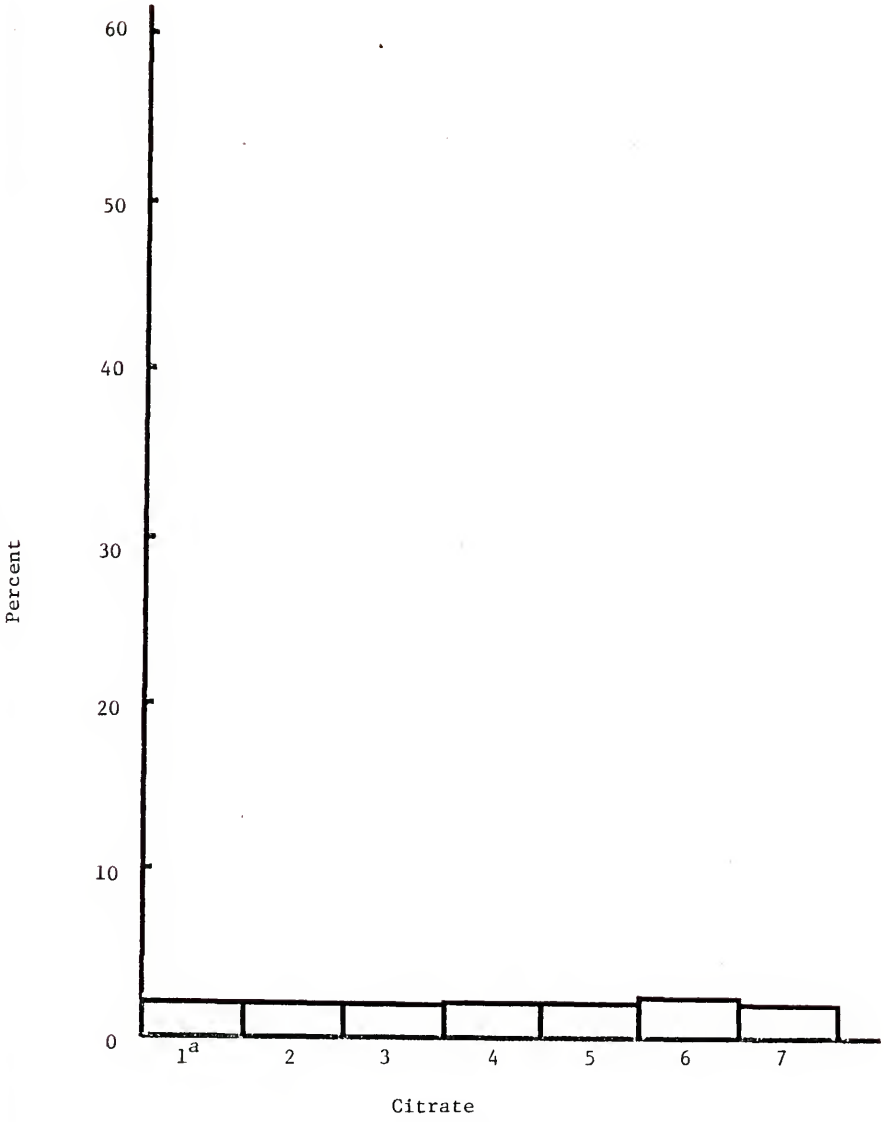

60
50
40
30
20
10
0
Percent
1[a]
2
3
4
5
6
7
Citrate

Figure 34. Percentages of the 18:0 fatty acid from *S. lactis* grown with various concentrations of citrate.

[a] 1 = no citrate; 2 = 0.0075 mM diammonium citrate; 3 = 0.075 mM diammonium citrate; 4 = 0.75 mM diammonium citrate; 5 = 7.5 mM diammonium citrate; 6 = 75.0 mM diammonium citrate; 7 = 7.5 mM diammonium phosphate.

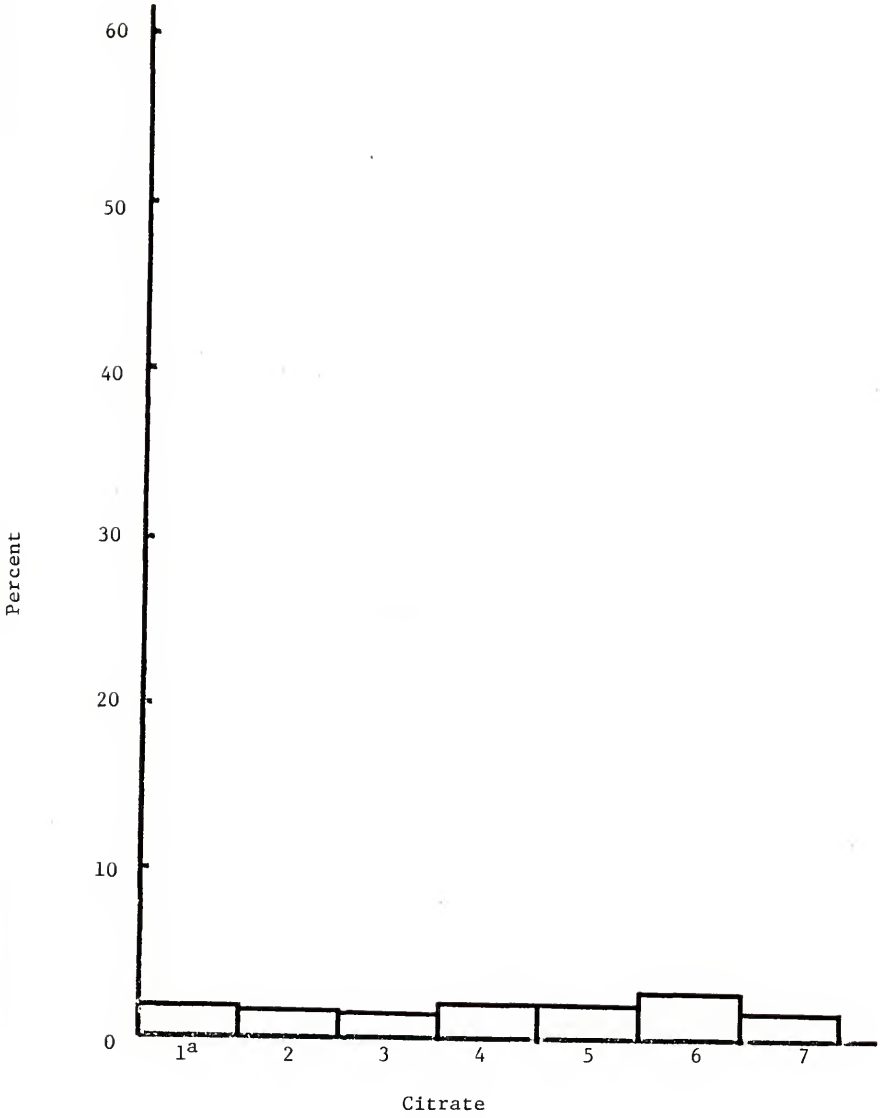

60
50
40
30
20
10
0
Percent
1a
2
3
4
5
6
7
Citrate

Figure 35. Percentages of the 18:1 fatty acid from *S. lactis* grown with various concentrations of citrate.

[a] 1 = no citrate; 2 = 0.0075 mM diammonium citrate; 3 = 0.075 mM diammonium citrate; 4 = 0.75 mM diammonium citrate; 5 = 7.5 mM diammonium citrate; 6 = 75.0 mM diammonium citrate; 7 = 7.5 mM diammonium phosphate.

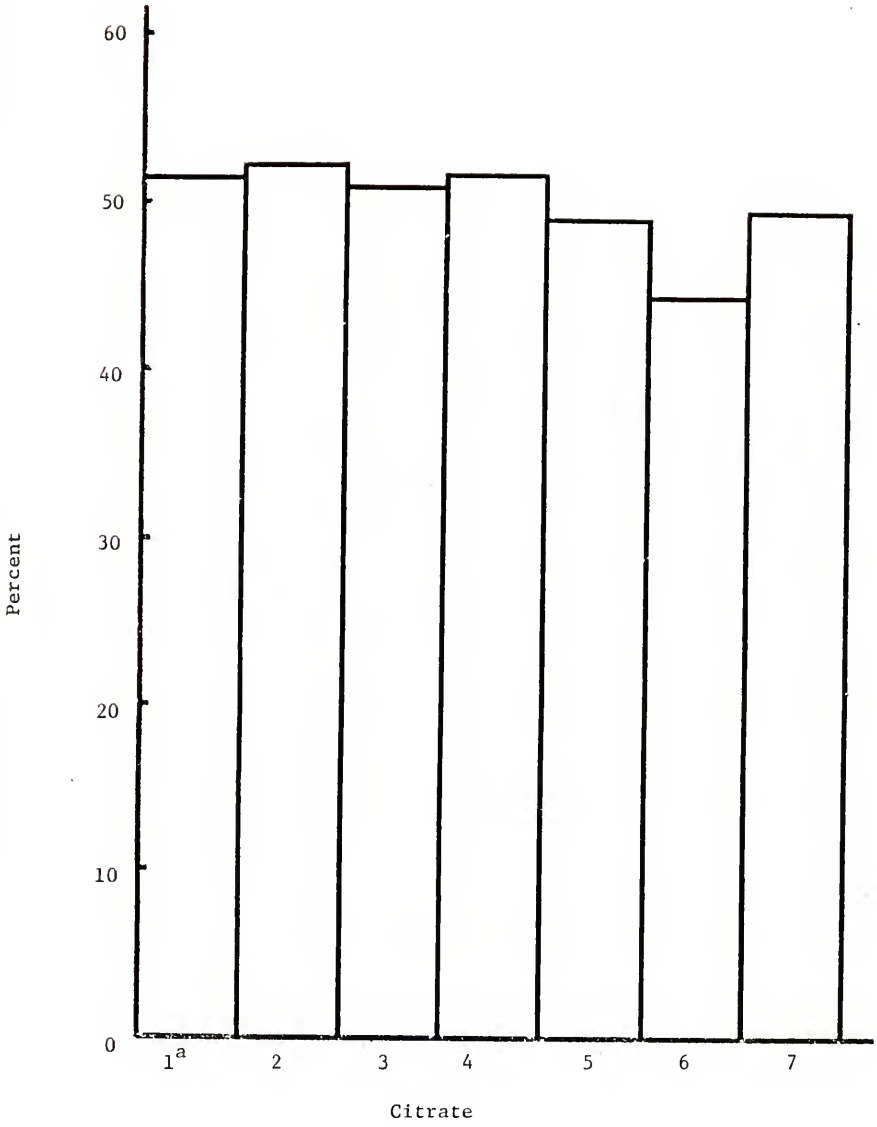

60
50
40
30
20
10
0
Percent
1[a]
2
3
4
5
6
7
Citrate

Figure 36. Percentages of the Δ19:0 fatty acid from *S. lactis* grown with various concentrations of citrate.

[a] 1 = no citrate; 2 = 0.0075 mM diammonium citrate; 3 = 0.075 mM diammonium citrate; 4 = 0.75 mM diammonium citrate; 5 = 7.5 mM diammonium citrate; 6 = 75.0 mM diammonium citrate; 7 = 7.5 mM diammonium phosphate.

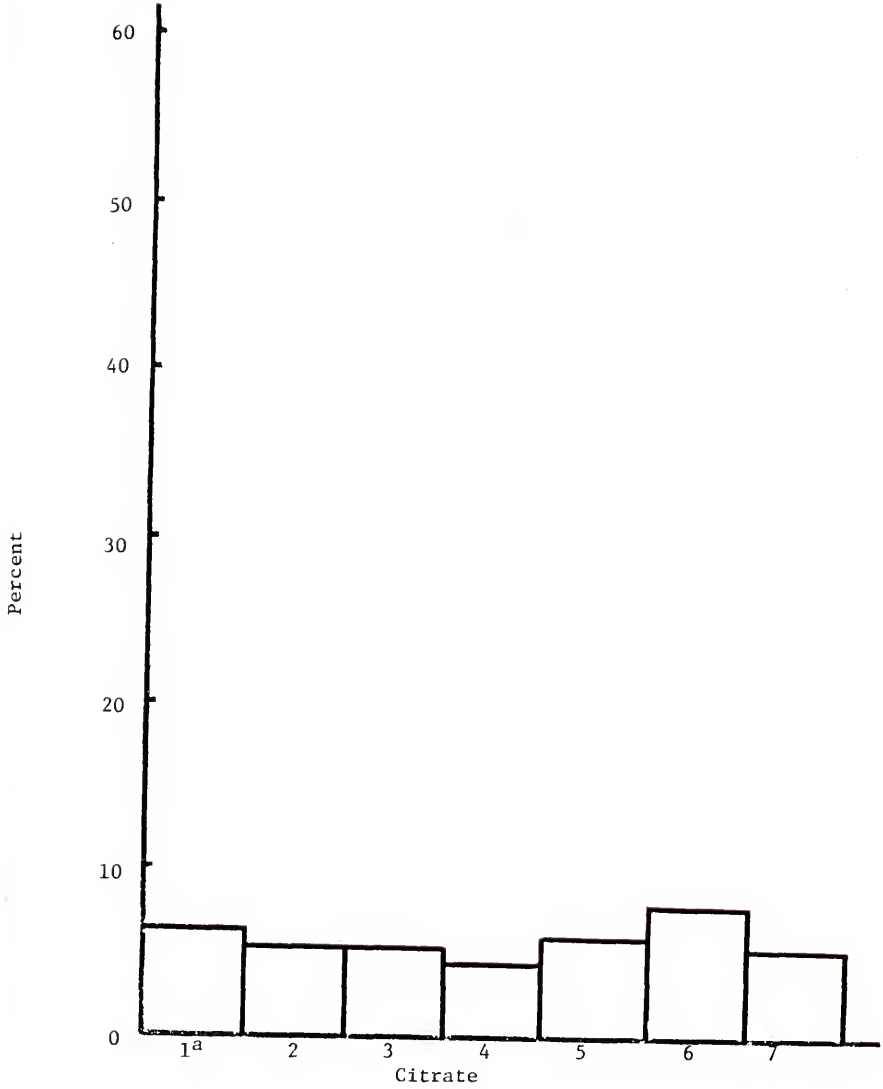

60
50
40
30
20
10
0
Percent
1[a]
2
3
4
5
6
7
Citrate

Figure 37. Percentages of the 21:0 fatty acid from *S. lactis* grown with various concentrations of citrate.

[a] 1 = no citrate; 2 = 0.0075 mM diammonium citrate; 3 = 0.075 mM diammonium citrate; 4 = 0.75 mM diammonium citrate; 5 = 7.5 mM diammonium citrate; 6 = 75.0 mM diammonium citrate; 7 = 7.5 mM diammonium phosphate.

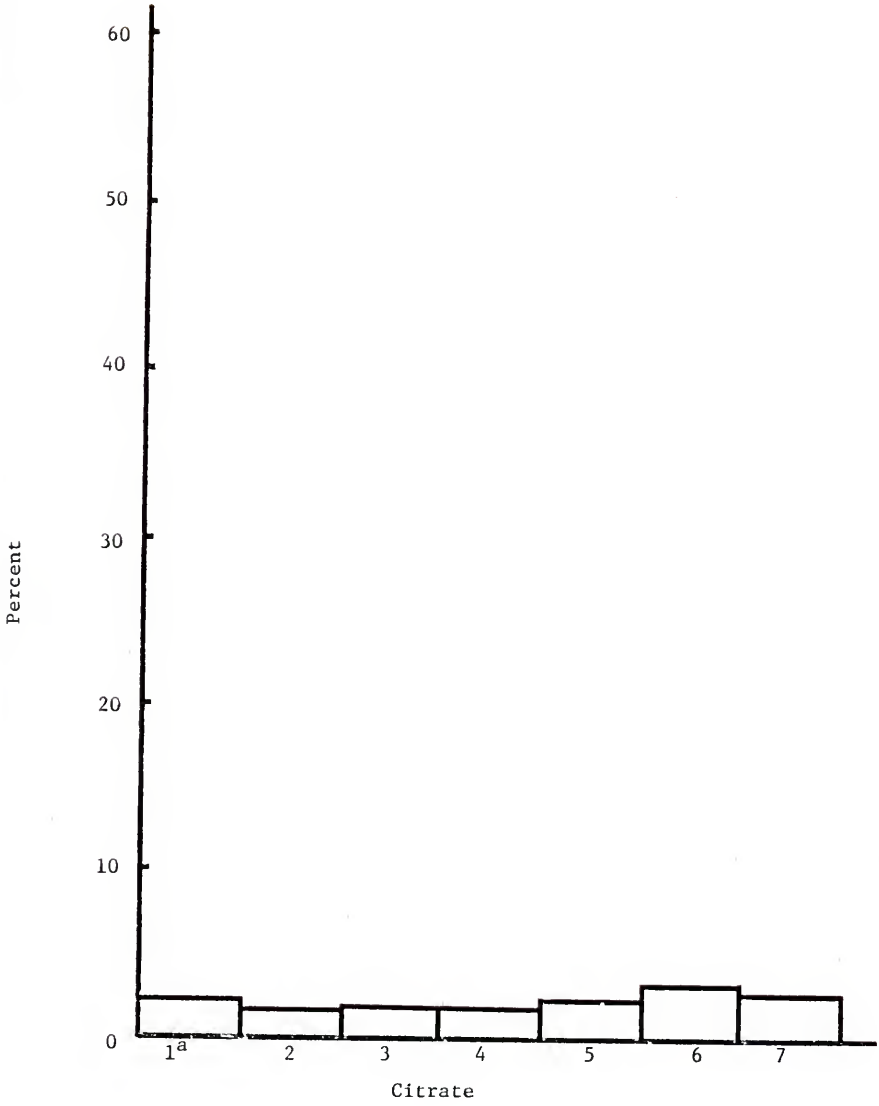

60
50
40
30
20
10
0
Percent
1[a]
2
3
4
5
6
7
Citrate

Table 17. F-values and significance of differences for citrate experiment.

| Fatty acid | F-value[a] | | | Significance[b] | | |
|---|---|---|---|---|---|---|
| | 1-6[c] | 1&7 | 5&7 | 1-6 | 1&7 | 5&7 |
| 14:0 | 3.64 | 0.74 | 1.00 | * | NS | NS |
| 16:0 | 0.53 | 1.35 | 0.40 | NS | NS | NS |
| 16:1 | 1.55 | 0.06 | 0.02 | NS | NS | NS |
| 18:0 | 11.01 | 13.50 | 8.91 | * | * | * |
| 18:1 | 30.56 | 2.19 | 0.16 | * | NS | NS |
| Δ19:0 | 4.50 | 1.23 | 0.97 | * | NS | NS |
| 21:0 | 6.70 | 0.43 | 0.35 | * | NS | NS |

[a] $F_{.05,5,12} = 3.1$; $F_{.05,1,4} = 7.71$

[b] * = significant; NS = not significant

[c] 1 = no citrate added; 2 = 0.0075 mM diammonium citrate; 3 = 0.075 mM diammonium citrate; 4 = 0.75 mM diammonium citrate; 5 = 7.5 mM diammonium citrate; 6 = 75.0 mM diammonium citrate; 7 = 7.5 mM diammonium phosphate.

## Discussion

The analysis of variance shows five fatty acids demonstrating significant differences when the different concentrations of citrate are compared. Those differences in 14:0, 18:0 and 21:0 fatty acids are extremely small numerically (<1 percentage point) and probably represent no functional significance. On the other hand, the 18:1 and Δ19:0 fatty acids again demonstrated the most dramatic differences in this experiment, though they are much less dramatic relative to the previous experiments described. The only significant differences in the controls were in the levels of the 18:0 fatty acid, but again they probably do not represent any functional significance due to their relatively low numerical values.

What is unexpected is the high level of the Δ19:0 fatty acid associated with the highest concentration of citrate. According to the literature, citrate was thought to chelate $Mg^{++}$, a cofactor required for cyclopropane fatty acid synthesis in *E. coli* (29). If this were true, the levels of the Δ19:0 fatty acid should have been decreased with the higher levels of citrate. Since these results are exactly opposite to those seen with *E. coli*, one must assume that another mechanism is probably at work.

If the results are again examined with respect to the proposed hypothesis of Δ19:0 fatty acid being highest when the organism is under duress, then the results make sense. Up to 7.5 mM, which is the normal concentration of citrate added to the medium, both the levels of 18:1 and Δ19:0 fatty acids deviate very little from the norm, and the rates of growth are also about the same. At a concentration of 75.0 mM, ten times the normal concentration added to the medium, the 18:1 content is down, the Δ19:0 content is up, and the growth rate is slower.

# CHAPTER VI
# EFFECTS OF ADDED FERMENTATION PRODUCTS ON THE FATTY ACID COMPOSITION OF *S. lactis*

## Literature Review

Since many bacteria such as *S. lactis* produce fermentation products as a result of growth, and since these products become a part of the growth environment, the effects of these compounds should not be ignored.

Fermentation of glucose, lactose or galactose in lactic streptococci is for the most part homofermentative resulting in lactic acid (32). Certain mutant strains as well as wild types grown on other carbohydrate sources are capable of producing a variety of fermentation products such as formic acid, acetic acid, acetoin, acetaldehyde, ethanol, diacetyl and carbon dioxide. In one study using *S. lactis* mutants, the maximum levels of acetic acid, formic acid and carbon dioxide produced were 0.07%, 0.06% and 0.04% respectively. These same mutants produced only trace amounts of ethanol while no values for the levels of acetoin or diacetyl were given. The wild type, on the other hand, produced 1.0% lactic acid as a maximum, demonstrating that even though other fermentation products are possible, their levels would be rather insignificant compared to normal lactic acid levels (13).

But are they insignificant when it comes to the effects on the cell membrane? Ingram (20) investigated the effects of acetaldehyde, acetic acid and ethanol on the fatty acid composition of *E. coli*. Acetalde-

hyde, up to concentrations of 0.1%, increased 16:0 fatty acid from 24.7 to 29.0%, decreased 16:1 fatty acid from 33.7 to 18.2%, decreased 18:1 fatty acid from 32.5 to 20.9% while increasing the Δ17:0 and Δ19:0 levels 4 percentage points and 8 percentage points respectively. With concentrations of acetic acid up to 2.4%, the only significant change occurred in the 16:1 fatty acid, a decrease from 37.4 to 27.2%. Ethanol at 4.0% decreased 16:0 fatty acid from 24.0 to 11.5% while increasing 18:1 fatty acid from 36.0 to 50.9%.

## Additional Methods

Cells were grown in the presence of the following concentrations of added fermentation products: no addition, 0.1% lactic acid, 1.0% lactic acid, 0.1% formic acid, 1.0% formic acid, 0.1% acetic acid, 1.0% acetic acid, 0.1% ethanol, 1.0% ethanol, 0.01% acetaldehyde, and 0.1% acetaldehyde.

Growth curves were used to determine doubling times. This experiment was done in triplicate.

## Results

Doubling times are shown in Table 18. The control plus the lower concentrations of lactic, formic and acetic acids, and ethanol demonstrated about the same growth rates. The 1.0% ethanol concentration seemed to stimulate growth. Slower growth resulted from the higher concentrations of lactic, formic and acetic acids, as well as from both concentrations of acetaldehyde.

Fatty acid percentages for each fermentation product tested are listed in Table 19. The 16:1 and 18:0 fatty acids remained relatively constant. The 14:0 content remained constant except at the higher

Table 18. Doubling times of *S. lactis* grown with various fermentation products added.

| Fermentation product | Doubling time (min.) |
|---|---|
| none | 90 |
| 0.1% Lactic acid | 90 |
| 1.0% Lactic acid | 103 |
| 0.1% Formic acid | 90 |
| 1.0% Formic acid | 117 |
| 0.1% Acetic acid | 90 |
| 1.0% Acetic acid | 117 |
| 0.1% Ethanol | 93 |
| 1.0% Ethanol | 78 |
| 0.01% Acetaldehyde | 108 |
| 0.1% Acetaldehyde | 223 |

Table 19. Fatty acid percentages from *S. lactis* grown with various fermentation products added.

| Fatty acid | Fermentation product | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1[a] | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 14:0 | 4.1 | 4.2 | 3.8 | 3.2 | 1.4 | 3.3 | 1.9 | 4.1 | 4.2 | 4.2 | 3.9 |
| 16:0 | 34.1 | 33.3 | 33.3 | 32.7 | 28.3 | 33.4 | 28.9 | 33.8 | 31.3 | 35.5 | 33.0 |
| 16:1 | 3.0 | 2.8 | 2.1 | 2.2 | 2.0 | 2.1 | 2.0 | 2.6 | 2.2 | 2.7 | 2.3 |
| 18:0 | 1.6 | 1.7 | 2.9 | 1.8 | 2.7 | 2.2 | 2.2 | 1.5 | 1.6 | 2.6 | 2.5 |
| 18:1 | 47.1 | 47.3 | 38.3 | 45.7 | 23.0 | 45.0 | 37.5 | 47.4 | 46.6 | 39.7 | 39.1 |
| Δ19:0 | 8.5 | 8.8 | 15.8 | 12.2 | 35.1 | 11.0 | 22.0 | 9.3 | 11.7 | 13.6 | 16.3 |
| 21:0 | 1.0 | 1.0 | 1.6 | 1.6 | 4.9 | 1.3 | 2.6 | 1.0 | 1.4 | 1.0 | 1.3 |

[a] 1 = no fermentation product added; 2 = 0.1% lactic acid; 3 = 1.0% lactic acid; 4 = 0.1% formic acid; 5 = 1.0% formic acid; 6 = 0.1% acetic acid; 7 = 1.0% acetic acid; 8 = 0.1% ethanol; 9 = 1.0% ethanol; 10 = 0.01% acetaldehyde; 11 = 0.1% acetaldehyde.

concentrations of formic and acetic acids where a decrease of about 2 percentage points occurred. For each product except lactic acid, the 16:0 content decreased about 4 percentage points with the higher concentration of that product. The 21:0 fatty acid maintained very low levels except when subjected to the higher concentrations of formic and acetic acids.

Again the 18:1 and Δ19:0 fatty acids showed the largest differences. With the lower concentrations of lactic, formic and acetic acids, and both concentrations of ethanol, the 18:1 content was the same as the control. The higher concentrations of lactic and acetic acids depressed the 18:1 content by about 9 percentage points while the higher concentrations of formic acid depressed it by about 23 percentage points. Both concentrations of acetaldehyde resulted in the same content of 18:1 fatty acid which was about 7 percentage points lower than the control.

In all cases of the Δ19:0 fatty acid, the higher levels of fermentation products caused higher percentages of the fatty acid as compared to the lower levels. Lower concentrations of lactic, formic and acetic acids, and ethanol resulted in Δ19:0 levels about the same as the control. Higher levels of lactic and acetic acids gave levels of Δ19:0 fatty acid which were twice that of the lower concentrations of those products. The higher level of formic acid gave a level of Δ19:0 fatty acid which was about three times the level of the lower concentration. Both concentrations of acetaldehyde resulted in levels of Δ19:0 fatty acid being higher than the control. Figures 38-44 demonstrate these results graphically.

Figure 38. Percentages of the 14:0 fatty acid from *S. lactis* grown with various added fermentation products.

[a] 1 = no added fermentation product; 2 = 0.1% lactic acid; 3 = 1.0% lactic acid; 4 = 0.1% formic acid; 5 = 1.0% formic acid; 6 = 0.1% acetic acid; 7 = 1.0% acetic acid; 8 = 0.1% ethanol; 9 = 1.0% ethanol; 10 = 0.01% acetaldehyde; 11 = 0.1% acetaldehyde.

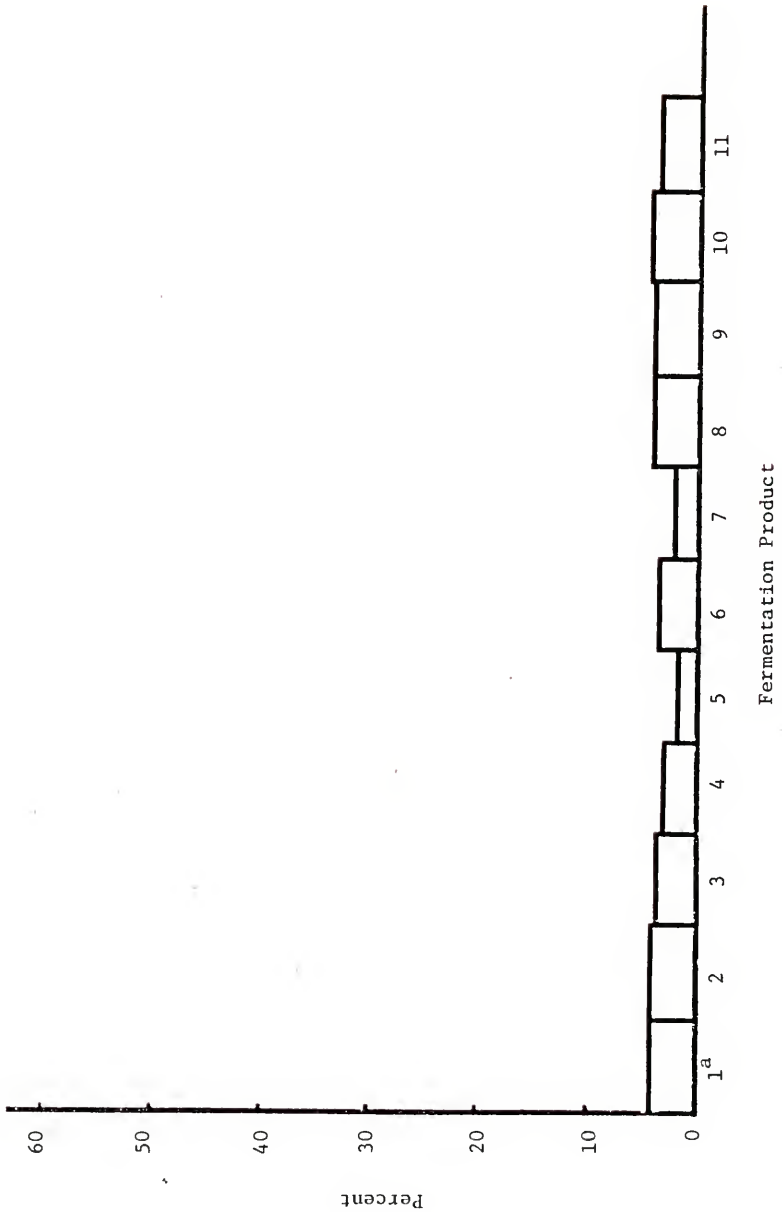

Percent
60
50
40
30
20
10
0
1[a]
2
3
4
5
6
7
8
9
10
11
Fermentation Product

Figure 39. Percentages of the 16:0 fatty acid from *S. lactis* grown with various added fermentation products.

[a] 1 = no added fermentation product; 2 = 0.1% lactic acid; 3 = 1.0% lactic acid; 4 = 0.1% formic acid; 5 = 1.0% formic acid; 6 = 0.1% acetic acid; 7 = 1.0% acetic acid; 8 = 0.1% ethanol; 9 = 1.0% ethanol; 10 = 0.01% acetaldehyde; 11 = 0.1% acetaldehyde.

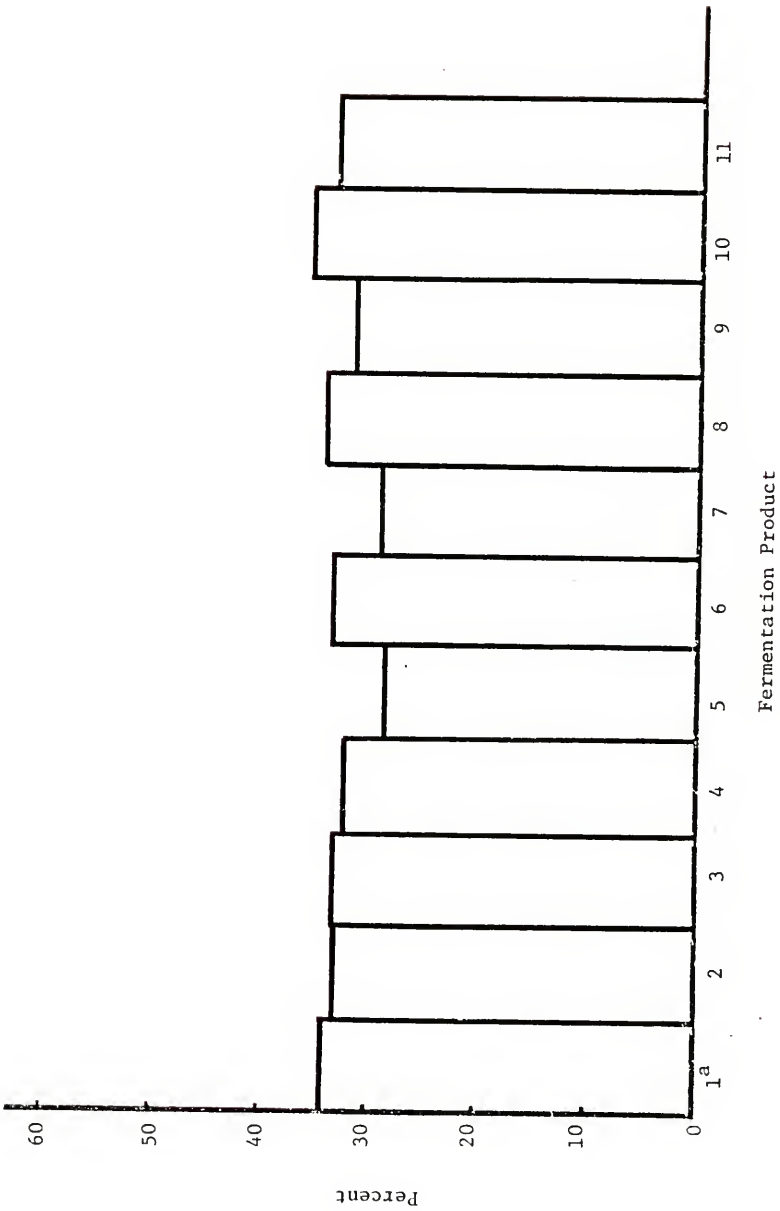

Percent
60
50
40
30
20
10
0
1[a]
2
3
4
5
6
7
8
9
10
11
Fermentation Product

Figure 40. Percentages of the 16:1 fatty acid from *S. lactis* grown with various added fermentation products.

[a] 1 = no added fermentation product; 2 = 0.1% lactic acid; 3 = 1.0% lactic acid; 4 = 0.1% formic acid; 5 = 1.0% formic acid; 6 = 0.1% acetic acid; 7 = 1.0% acetic acid; 8 = 0.1% ethanol; 9 = 1.0% ethanol; 10 = 0.01% acetaldehyde; 11 = 0.1% acetaldehyde.

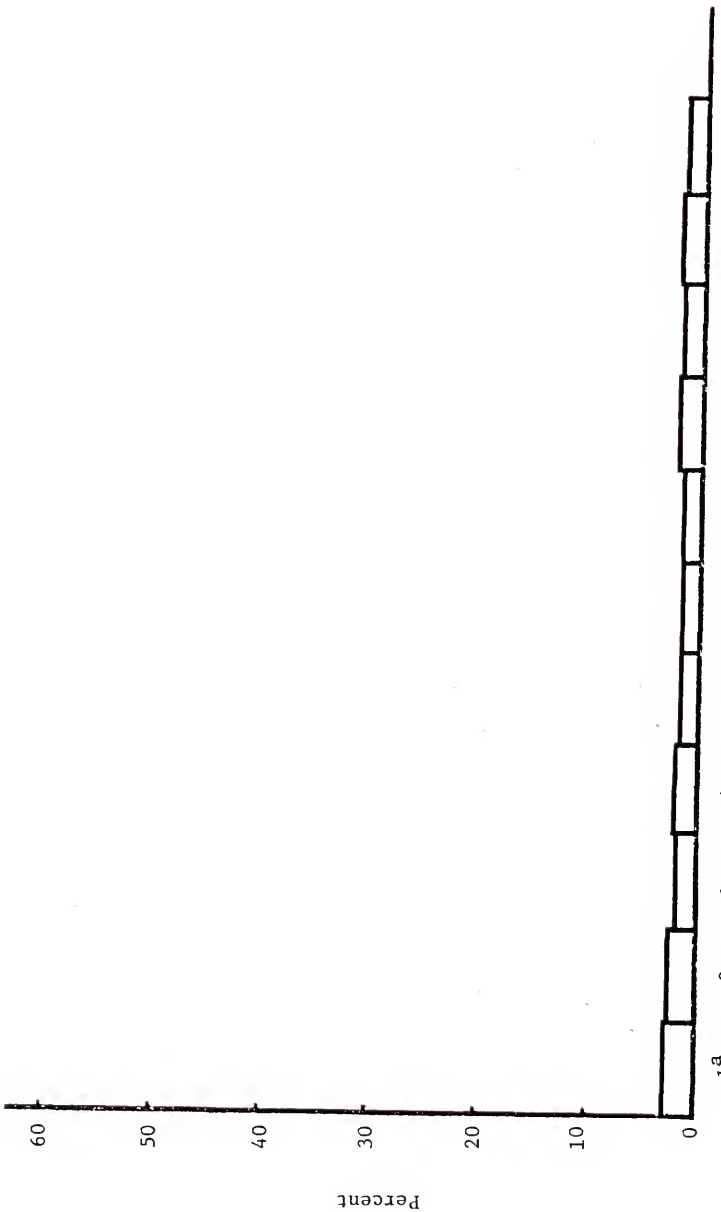

Percent
60
50
40
30
20
10
0
1[a]
2
3
4
5
6
7
8
9
10
11
Fermentation Product

Figure 41. Percentages of the 18:0 fatty acid from *S. lactis* grown with various added fermentation products.

[a] 1 = no added fermentation product; 2 = 0.1% lactic acid; 3 = 1.0% lactic acid; 4 = 0.1% formic acid; 5 = 1.0% formic acid; 6 = 0.1% acetic acid; 7 = 1.0% acetic acid; 8 = 0.1% ethanol; 9 = 1.0% ethanol; 10 = 0.01% acetaldehyde; 11 = 0.1% acetaldehyde.

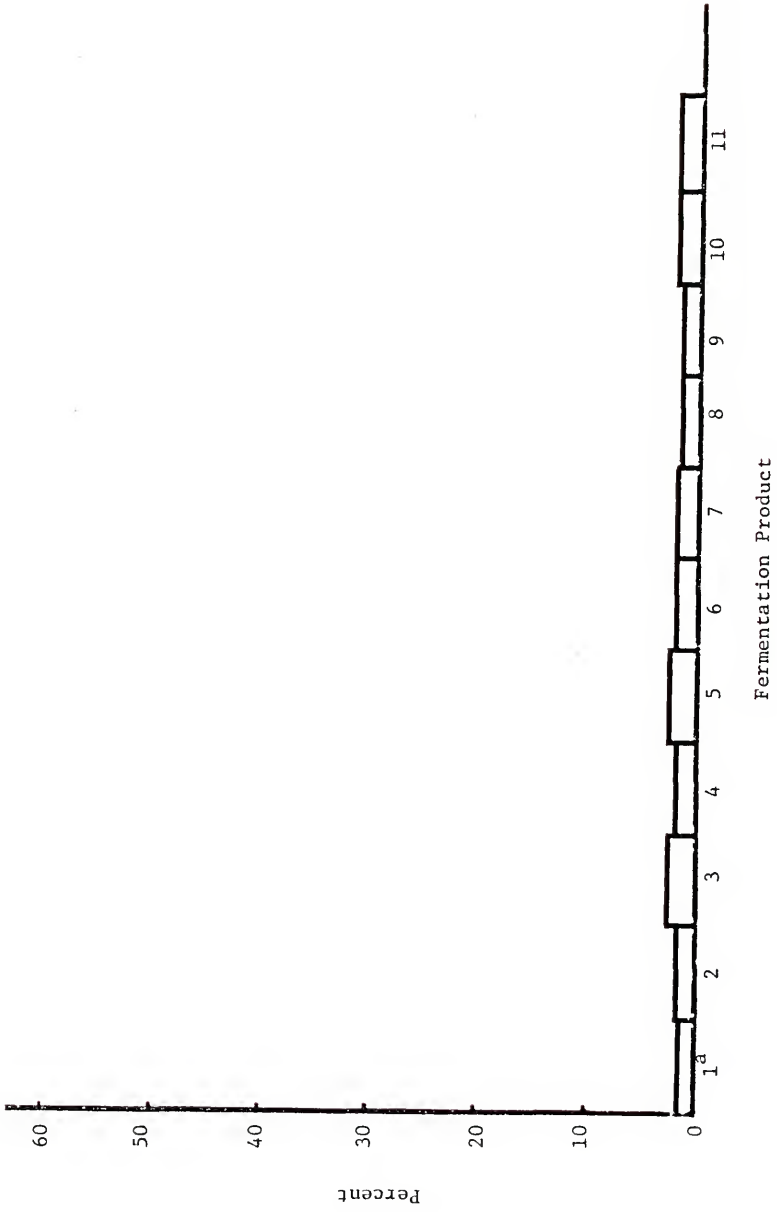

Percent
60
50
40
30
20
10
0
1[a]
2
3
4
5
6
7
8
9
10
11
Fermentation Product

Figure 42. Percentages of the 18:1 fatty acid from *S. lactis* grown with various added fermentation products.

[a] 1 = no added fermentation product; 2 = 0.1% lactic acid; 3 = 1.0% lactic acid; 4 - 0.1% formic acid; 5 = 1.0% formic acid; 6 = 0.1% acetic acid; 7 = 1.0% acetic acid; 8 = 0.1% ethanol; 9 = 1.0% ethanol; 10 = 0.01% acetaldehyde; 11 = 0.1% acetaldehyde.

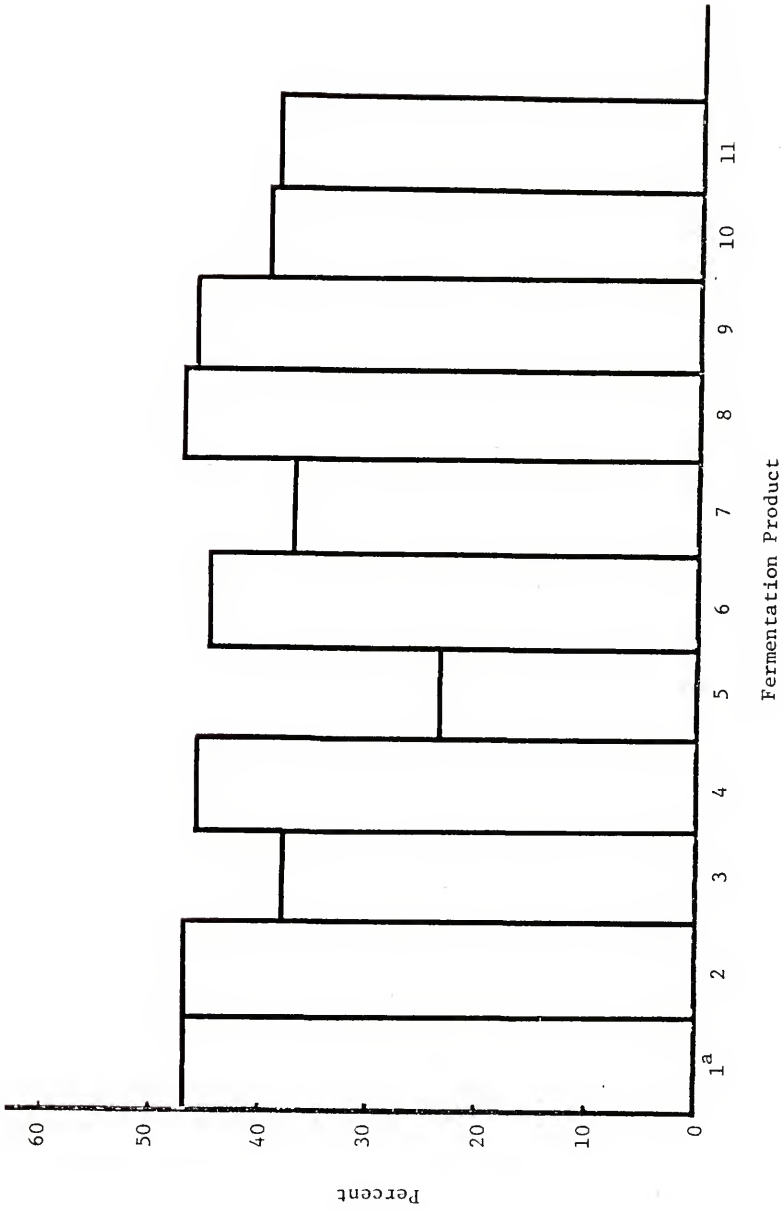

Percent
60
50
40
30
20
10
0
1a
2
3
4
5
6
7
8
9
10
11
Fermentation Product

Figure 43. Percentages of the Δ19:0 fatty acid from *S. lactis* grown with various added fermentation products.

[a] 1 = no added fermentation product; 2 = 0.1% lactic acid; 3 = 1.0% lactic acid; 4 = 0.1% formic acid; 5 = 1.0% formic acid; 6 = 0.1% acetic acid; 7 = 1.0% acetic acid; 8 = 0.1% ethanol; 9 = 1.0% ethanol; 10 = 0.01% acetaldehyde; 11 = 0.1% acetaldehyde.

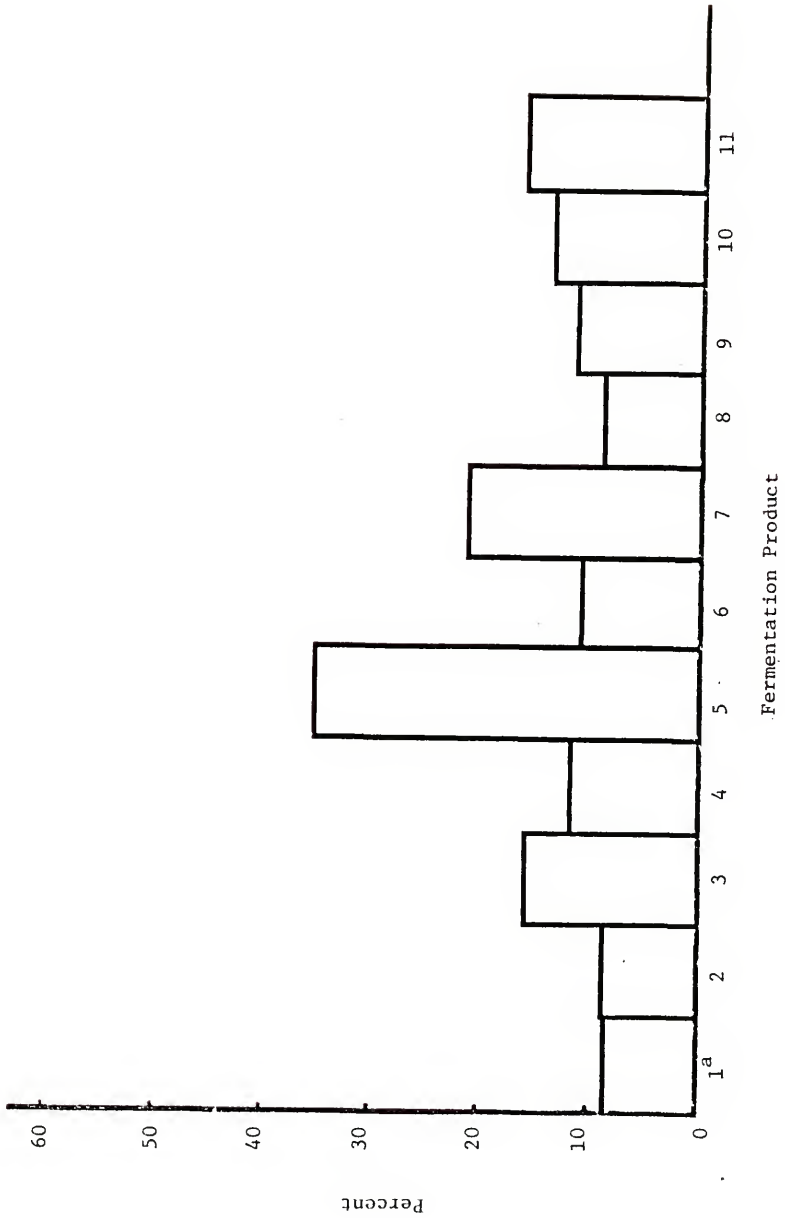
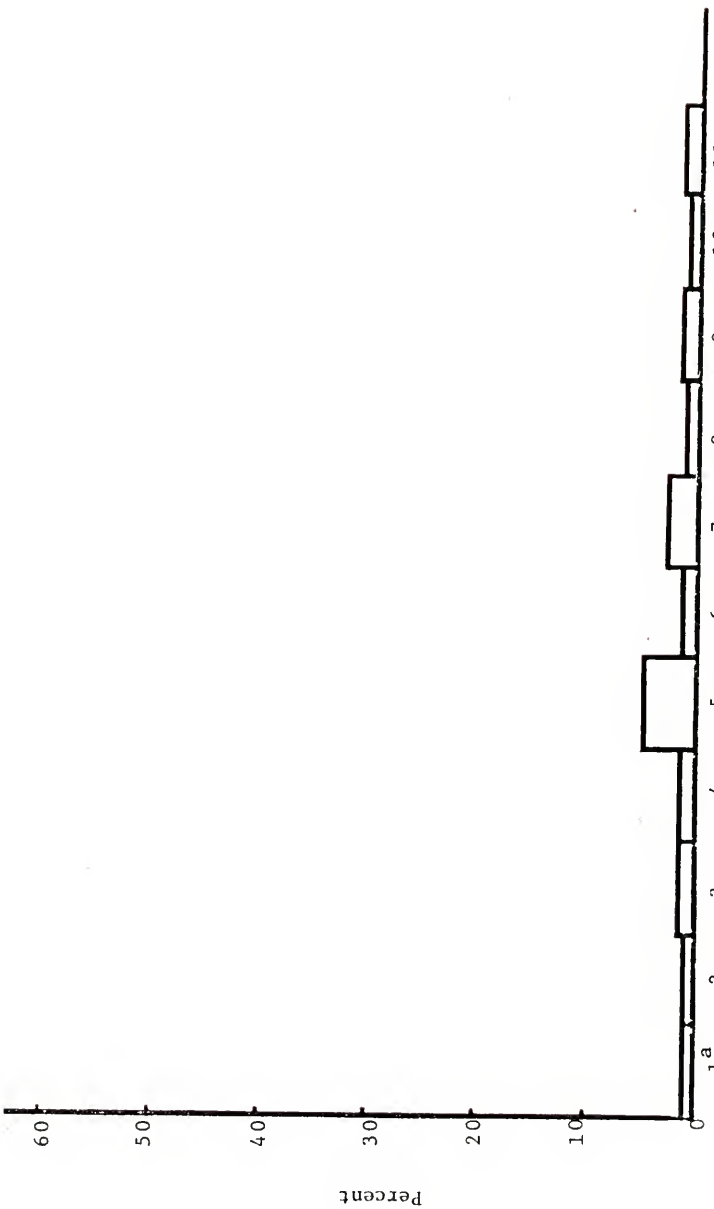

Percent
60
50
40
30
20
10
0
1[a]
2
3
4
5
6
7
8
9
10
11
Fermentation Product

Figure 44. Percentages of the 21:0 fatty acid from *S. lactis* grown with various added fermentation products.

[a] 1 = no added fermentation product; 2 = 0.1% lactic acid; 3 = 1.0% lactic acid; 4 = 0.1% formic acid; 5 = 1.0% formic acid; 6 = 0.1% acetic acid; 7 = 1.0% acetic acid; 8 = 0.1% ethanol; 9 = 1.0% ethanol; 10 = 0.01% acetaldehyde; 11 = 0.1% acetaldehyde.


Percent
60
50
40
30
20
10
0
1a
2
3
4
5
6
7
8
9
10
11
Fermentation Product

## Discussion

Again the most striking differences occur in the 18:1 and Δ19:0 fatty acids. An analysis of the differences observed in the 18:1 content shows the lower percentages to be associated with the higher concentrations of each fermentation product tested. The opposite is true for the Δ19:0 fatty acid. The higher concentration of each fermentation product is associated with the higher percentage of the Δ19:0 fatty acid. Again the more adverse conditions, associated with the higher fermentation product concentrations, are resulting in decreased levels of 18:1 fatty acid and increased levels of Δ19:0 fatty acid.

## GENERAL DISCUSSION

The membrane fatty acid composition of *S. lactis* ATCC 12929 varies significantly during changes in temperature, pH, growth phase, carbohydrate source, presence of various levels of citrate and presence of added fermentation products.

Of the seven major fatty acids, the 18:1 and Δ19:0 fatty acids are the only ones demonstrating both highly significant differences as well as relatively large numerical differences. Differences in these two fatty acids seem to be associated with growth conditions. When the organism would seem to be most content, which is not necessarily the same as most rapidly growing, the levels of 18:1 fatty acid are the highest and the levels of Δ19:0 fatty acid are the lowest in most cases. As growth conditions become more adverse, the Δ19:0 levels increase at the expense of a decrease in the 18:1 levels. Overall, there does seem to be a gross relationship between conversion of 18:1 to Δ19:0 fatty acid and rate of growth, but mathematical models tested have failed to demonstrate any quantitative correlations.

So what role does the cyclopropane fatty acid play in this adjustment to adverse conditions. Is it required for cell survival? In *E. coli*, it has been shown that cyclopropane fatty acid synthesis is not required for cell survival since unsaturated fatty acid auxotrophs grow on trans-unsaturated fatty acids with virtually no cyclopropane fatty acids synthesized (43). Taylor and Cronan (46) demonstrated growth of a

cyclopropane fatty acid-deficient mutant of *E. coli* without supplementation of the cyclopropane fatty acid. There is also a mutant of *S. faecalis* which cannot synthesize cyclopropane fatty acids, yet it demonstrates viability in its absence (21,22).

One proposed role for cyclopropane fatty acids has been that of prevention of peroxidation of unsaturated fatty acids (31). But studies done with *E. coli* and *P. fluorescens* showed that oxygenation of the medium actually decreased cyclopropane fatty acid production by the cells (9,28).

A second proposal was that cyclopropane fatty acid synthesis might decrease the concentration of such metabolic products as S-adenosylmethionine and unsaturated fatty acids. However, Cronan, et al. (7) showed that cyclopropane fatty acid synthesis is not controlled by the levels of S-adenosylmethionine or unsaturated fatty acids.

In 1968 Cronan (5) suggested that the conversion of unsaturated fatty acids to cyclopropane fatty acids on phospholipid molecules might serve to protect the phospholipids from degradation during stationary phase. This hypothesis was based on the observation that phospholipid content does not decrease during stationary phase while stationary phase cells have appreciable phospholipase activity and less than 1% of the phospholipid biosynthetic rate of growing cells (5,45). However, in 1974 Cronan et al. (7) demonstrated that the amount of cyclopropane fatty acid in a phospholipid molecule does not seem to affect the stability of that molecule.

There is another possible role for the formation of cyclopropane fatty acids. It is well known that the degree of saturation of *E. coli* phospholipids increases with aging of cells and with increasing temper-

ature (28). It is also known that cyclopropane fatty acids increase under the same conditions that increase saturates and decrease unsaturates (28). Apparently this increase in saturates is a membrane fluidity adjustment in response to the adverse conditions associated with aging and high temperatures. The increased saturates may be acting to add some sort of protection to the membrane. If cyclopropane fatty acids are taken as intermediate between saturated and unsaturated as pertains to lipid phase characteristics, rather than as purely unsaturates (28), then the increase in cyclopropane fatty acids found associated with adverse conditions may further increase the stability of the membrane.

A protective effect due to cyclopropanes seems to be evident in organisms other than bacteria. In the female millipede, *Graphidostreptis tumuliporus,* there is a correlation between egg-ripening and the synthesis of cyclopropane fatty acids. The function of the cyclopropanes in millipedes is concerned with the maintenance of the lipid structure of the early larval egg which is vulnerable to its desiccating and arid environment (38). In leaves of snowdrops, *Galanthus nivalis,* and cow parsley, *Anthriscus silvestris,* the content of cyclopropane fatty acids appears to be related to the capacity of the plants to grow at low temperature. These fatty acids are believed to allow increased mobility of the hydrocarbon chains in the membrane at low temperature compared to the saturated analog (30).

If the cyclopropane fatty acids do exhibit fluidity characteristics between those of saturates and unsaturates, then these fatty acids may be functioning in a dual capacity. First, they may be providing a physicochemical fluidity comparable to the unsaturated analog, thus allowing function of membrane components which require a fluid state. Secondly,

they may be stabilizing the membrane as saturates do, thus affording some protection during adverse conditions.

Taylor and Cronan (46) believe that a paradox exists. They studied *E. coli* cyclopropane fatty acid-deficient mutants which failed to demonstrate a physiological phenotype. Under all conditions tested, they behaved like the parental strain, raising the question of why cyclopropane fatty acids are formed. One suggestion was that a proper stress to reveal the function of these acids may not yet have been found. They also suggested that synthesis of cyclopropane fatty acids may be metabolically expensive, since it requires S-adenosylmethionine as methyl donor. But if S-adenosylmethionine is present in excess during late exponential phase (7), then the formation of cyclopropane fatty acids would require no additional energy expenditures. On the other hand, if it became necessary to synthesize additional quantities of S-adenosylmethionine, then the relatively small amount of energy required (one ATP per S-adensylmethionine molecule) may be well worth the expense in order to provide the dual function hypothesized for the cyclopropane fatty acids.

BIBLIOGRAPHY


1. Bretscher, M.S. 1973. Membrane structure: some general principles. Science *181*:622-629.

2. Capaldi, R.A. 1974. A dynamic model of cell membranes. Sci. Am. *230*:26-33.

3. Carroll, K.K. 1961. Quantitative estimation of peak areas in gas-liquid chromatography. Nature *191*:377-378.

4. Chapman, D. 1975. Lipid dynamics in cell membranes, p. 13-22. *In* G. Weissmann and R. Claiborne (ed.), Cell membranes: biochemistry, cell biology and pathology. H.P. Publishing Co., New York.

5. Cronan, J.E., Jr. 1968. Phospholipid alterations during growth of *Escherichia coli*. J. Bacteriol. *95*:2054-2061.

6. Cronan, J.E., Jr., and E.P. Gelmann. 1975. Physical properties of membrane lipids: biological relevance and regulation. Bacteriol. Rev. *39*:232-256.

7. Cronan, J.E., Jr., W.D. Nunn, and J.G. Batchelor. 1974. Studies on the biosynthesis of cyclopropane fatty acids in *Escherichia coli*. Biochim. Biophys. Acta *384*:63-75.

8. Cronan, J.E., Jr., and P.R. Vagelos. 1972. Metabolism and function of the membrane phospholipids of *Escherichia coli*. Biochim. Biophys. Acta *265*:25-60.

9. Crowfoot, P.D., and A.L. Hunt. 1970. The effect of oxygen tension on methylene hexadecanoic acid formation in *Pseudomonas fluorescens* and *Escherichia coli*. Biochim. Biophys. Acta *202*:550-552.

10. Cullen, J., M.C. Phillips, and G.G. Shipley. 1971. The effects of temperature on the composition and physical properties of the lipids of *Pseudomonas fluorescens*. Biochem. J. *125*:733-742.

11. Danielli, J.F. 1975. The bilayer hypothesis of membrane structure, p. 3-11. *In* G. Weissmann and R. Claiborne (ed.), Cell membranes: biochemistry, cell biology and pathology. H.P. Publishing Co., Inc. New York.

12. Deibel, R.H., and H.W. Seeley, Jr. 1974. Family II. *Streptococcaceae fam. nov.*, p. 507. *In* R.E. Buchanan and N.E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore, Maryland.

13. Demko, G.M., S.J.B. Blanton, and R.E. Benoit. 1972. Heterofermentative carbohydrate metabolism of lactose-impaired mutants of *Streptococcus lactis*. J. Bacteriol. *112*:1335-1345.

14. DeRosa, M., A. Gambacorta, and J.D. Bu'Lock. 1974. Effect of pH and temperature on the fatty acid composition of *Bacillus acidocaldarius*. J. Bacteriol. *117*:212-214.

15. Drucker, D.B., C.J. Griffith, and T.H. Melville. 1974. Fatty acid fingerprints of Streptococci: variability due to carbohydrate source. Microbios *9*:187-189.

16. Drucker, D.B., C.J. Griffith, and T.H. Melville. 1975. The effect of changing pH on fatty-acid profiles of *Streptococcus mutans* NCTC 10832. Microbios *13*:99-103.

17. Drucker, D.B., and F.J. Veazey. 1977. Fatty acid fingerprints of *Streptococcus mutans* NCTC 10832 grown at various temperatures. Appl. Environ. Microbiol. *33*:221-226.

18. Eisenberg, M., and S. McLaughlin. 1976. Lipid bilayers as models of biological membranes. Bioscience *26*:436-443.

19. Gill, C.O. 1975. Effect of growth temperature on the lipids of *Pseudomonas fluorescens*. J. Gen. Microbiol. *89*:293-298.

20. Ingram, L.O. 1976. Adaptation of membrane lipids to alcohols. J. Bacteriol. *125*:670-678.

21. Jungkind, D.L., and R.C. Wood. 1974. Factors involved in the synthesis of cyclopropane fatty acids by *Streptococcus faecalis*. Biochim. Biophys. Acta *337*:286-297.

22. Jungkind, D.L., and R.C. Wood. 1974. Physiological differences between cyclopropane fatty acid-deficient mutants and the parent strain of *Streptococcus faecalis*. Biochim. Biophys. Acta *337*:298-310.

23. Kaneda, T. 1977. Fatty acids of the genus Bacillus: an example of branch-chain preference. Bacteriol. Rev. *41*:391-418.

24. Kanfer, J., and E.P. Kennedy. 1963. Metabolism and function of bacterial lipids. J. Biol. Chem. *238*:2919-2922.

25. Kates, M. 1964. Bacterial lipids, p. 17-90. *In* R. Paoletti and D. Kritchevensky (ed.), Advances in lipid research, Vol. 2. Academic Press, Inc., New York.

26. Kates, M., G.A. Adams, and S.M. Martin. 1964. Lipids of *Serratia marcescens*. Can. J. Biochem. *42*:461-479.

27. Khuller, G.K., and H. Goldfine. 1974. Phospholipids of *Clostridium butyricum*. V. Effects of growth temperature on fatty acid, alk-1-enyl ether group, and phospholipid composition. J. Lipid Res. *15*:500-507.

28. Kito, M., M. Ishinago, S. Aibara, M. Kato, and T. Hata. 1973. Relation between cyclopropane fatty acid formation and saturation of phospholipid species in *Escherichia coli* K-12. Agr. Biol. Chem. *37*:2647-2651.

29. Knivett, V.A., and J. Cullen. 1965. Some factors affecting cyclopropane fatty acid formation in *Escherichia coli*. Biochem. J. *96*:771-776.

30. Kuiper, P.J.C., and B. Stuiver. 1972. Cyclopropane fatty acids in relation to earliness in spring and drought tolerance in plants. Plant Physiol. *49*:307-309.

31. Law, J.D., H. Zalkin, and T. Kaneshiro. 1963. Transmethylation reactions in bacterial lipids. Biochim. Biophys. Acta *70*:143-151.

32. Lawrence, R.C., T.D. Thomas, and B.E. Terzaghi. 1976. Reviews of the progress of dairy science: cheese starters. J. Dairy Res. *43*:141-193.

33. Machtiger, N.A., and C.F. Fox. 1973. Biochemistry of bacterial membranes. Ann. Rev. Biochem. *42*:575-600.

34. MacLeod, P., and J.P. Brown. 1963. Fatty acid composition of lipids from *Streptococcus cremoris* and *Streptococcus lactis* var. maltigenes. J. Bacteriol. *85*:1056-1060.

35. MacLeod, P., R.G. Jensen, G.W. Gander, and J. Sampugna. 1962. Quantity and fatty acid composition of lipid extracted from cells of *Streptococcus lactis*. J. Bacteriol. *83*:806-810.

36. Marr, A.G., and J.L. Ingraham. 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli*. J. Bacteriol. *84*:1260-1267.

37. O'Leary, W.M. 1962. S-adenosylmethionine in the biosynthesis of bacterial fatty acids. J. Bacteriol. *84*:967-972.

38. Oudejans, R.C.H.M., D.J. Van Der Horst, F.A. Opmaer, and W.J. Tieleman. 1976. On the function of cyclopropane fatty acids in millipedes (Diplopoda). Comp. Biochem. Physiol. *54B*:227-230.

39. Packter, N.M. 1973. Biosynthesis of acetate-derived compounds. John Wiley & Sons Ltd., London.

40. Reiter, B., and J.D. Oram. 1962. Nutritional studies on cheese starters. J. Dairy Res. *29*:63-77.

41. Saier, M.H., Jr., and C.D. Stiles. 1975. Molecular dynamics in biological membranes. Springer-Verlag Inc., New York.

42. Silbert, D.F., R.C. Ladenson, and J.L. Honegger. 1973. The unsaturated fatty acid requirement in *Escherichia coli*. Biochim. Biophys. Acta *311*:349-361.

43. Silbert, D.F., F. Ruch, and P.R. Vagelos. 1968. Fatty acid replacements in a fatty acid auxotroph of *Escherichia coli*. J. Bacteriol. *95*:1658-1665.

44. Singer, S.J., and G.L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. Science *175*:720-731.

45. Tarbov, A.R., and E.P. Kennedy. 1965. The β-galactoside permease system and the metabolism of phospholipids in *Escherichia coli*. J. Biol. Chem. *240*:49-53.

46. Taylor, F., and J.E. Cronan, Jr. 1976. Selection and properties of *Escherichia coli* mutants defective in the synthesis of cyclopropane fatty acids. J. Bacteriol. *125*:518-523.

47. Umemoto, Y., and Y. Sato. 1978. Fatty acid composition of various lipid fractions of *Streptococcus lactis* grown at low temperature. Agr. Biol. Chem. *42*:213-219.

48. Weerkamp, A., and W. Heinen. 1972. Effect of temperature on the fatty acid composition of the extreme thermophiles, *Bacillus caldolyticus* and *Bacillus caldotenax*. J. Bacteriol. *109*:443-446.

49. Wisnieski, B.J., J.G. Parkes, Y.O. Huang, and C.F. Fox. 1974. Physical and physiological evidence for two phase transitions in cytoplasmic membranes of animal cells. Proc. Nat. Acad. Sci. USA *71*:4381-4385.

50. Zalkin, H., J.H. Law, and H. Goldfine. 1963. Enzymatic synthesis of cyclopropane fatty acids catalyzed by bacterial extracts. J. Biol. Chem. *238*:1242-1248.

## BIBLIOGRAPHICAL SKETCH

Timothy Alan Kral was born on September 29, 1951, in Cleveland, Ohio. He received his first six years of formal education at Edward M. Williams Elementary School followed by two years at St. Henry's, a parochial grade school. He attended Padua Franciscan High School from 1965 through 1969, graduating with high honors. In 1969, he entered John Carroll University and graduated with honors in 1973 with a Bachelor of Science degree in biology. In 1973, he began graduate studies in the Department of Microbiology at the University of Florida working toward the degree of Doctor of Philosophy.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Kenneth L. Smith, Chairman
Associate Professor of
Dairy Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

K.C. Bachman
Associate Professor of
Dairy Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

A.S. Bleiweis
Professor of Microbiology and
Cell Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

P.H. Smith
Professor and Chairman of
Microbiology and Cell Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1978

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School

UNIVERSITY OF FLORIDA
3 1262 08553 2678